CHAPTER

5 Immunohematology

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I. IMMUNOHEMATOLOGY OVERVIEW

A. Definition: Immunohematology is the study of blood group antigens and antibodies, HLA antigens and antibodies, pretransfusion testing, identification of unexpected alloantibodies, immune hemolysis, autoantibodies, drugs, blood collection, blood components, cryopreservation of blood, transfusion-transmitted viruses, tissue banking and organ transplantation, blood transfusion practice, safety, quality assessment, records, blood inventory management, and blood usage review.

B. Immune System

1. **Acquired immunity** is a **specific response** of the immune system in which antibodies specific to a particular antigen are produced. **Plasma cells** produce **antibodies.**
2. **Innate immunity** is a **nonspecific reaction** of the immune system that attacks all invaders. It includes physical and biochemical barriers and cells such as leukocytes, including neutrophils, monocytes/macrophages, and natural killer cells. Physical barriers include intact skin, mucous membranes, etc. Bactericidal enzymes are biochemical barriers.

C. Antigen Characteristics

1. **Antigens** are substances that **combine with** an **antibody.** An **antigen** that causes a **specific immune response** is an **immunogen.** Immunogens are made of protein, carbohydrates, and combinations of both. Antigens are found on the surface of platelets and WBCs as well as RBCs. Some immunogens produce a greater response than others.
2. There are **23 RBC antigen systems** containing over 200 RBC antigens. RBC antigens are inherited and are composed of proteins, glycoproteins, and glycolipids.
3. **Human leukocyte antigens (HLAs)**
   a. Present on **leukocytes** and **tissue cells**
   b. **Genes** that encode the **HLA antigens** are part of the **major histocompatibility complex** (MHC).
   c. **MHC** is on chromosome 6 and is divided into **Class I, II,** and **III.**
      1) **Class I** includes A, B, and C loci.
      2) **Class II** includes DR, DP, and DQ.
      3) **Class III** includes complement proteins.
   d. Immune response to transfused incompatible HLA antigens causes fever and chills. This is known as a febrile, non-hemolytic transfusion reaction.
   e. **HLA** must be matched for **organ, tissue, bone marrow,** and **stem cell transplant** donors and recipients. If the recipient is not matched correctly, a severe **graft-versus-host disease** results.
   f. **HLA test applications** include paternity testing, organ and tissue transplantation, bone marrow and stem cell transplantation, and platelet matching.

4. **Platelet antigens**
   a. Membranes have **protein antigens.**
   b. **Platelet antibodies** occur less frequently in the general population because of less antigen variability.
   c. Antibodies reacting with platelets may be ABO-, HLA-, or platelet specific.
   d. **Diseases:** Neonatal alloimmune thrombocytopenia and posttransfusion purpura

D. Antibody Characteristics

1. **Molecular structure**
   a. Each molecule has **two heavy** chains and **two light** chains.
   b. The **heavy chain** is responsible for the immunoglobulin **group specificity.**
   c. **Antibody binding site** is found in the **variable region** of the heavy and light chains.
2. **IgM antibodies**
   a. Composed of five basic immunoglobulin units (pentamer)
   b. Can directly bind with RBCs and produce agglutination
   c. Can activate complement
   d. Cannot cross the placenta because of large size of molecule
   e. React optimally at room temperature and below
   f. Usually clinically insignificant
3. **IgG antibodies**
   a. Single immunoglobulin unit
   b. Cannot visibly agglutinate RBCs
   c. Normally, cannot activate complement unless two molecules are present (i.e., IgG3)
   d. Can cross the placenta
   e. React optimally at 37°C
   f. Typically clinically significant; capable of causing transfusion reactions or hemolytic disease of the newborn (HDN)

E. Antigen-Antibody Interactions

1. Follow the Law of Mass Action
2. Reversible
3. Antigen-antibody complex formed
4. Properties that influence antigen-antibody interactions:
   a. Fit of antigen into antibody binding site
   b. Size of antigen
   c. Shape of antigen
   d. Charge of antigen
5. **Antigen-antibody complexes** are held together by electrostatic charges, hydrogen bonding, hydrophobic bonding, and Van der Waals forces.

F. Antigen-Antibody Reactions *In Vivo*

1. **Transfusions** can lead to antigen-antibody complex formation and complement activation *in vivo,* if wrong type of blood is transfused.
2. Transfusion of foreign antigens (RBC, HLA, and platelet) into a recipient can cause an immune response and antibody formation in the recipient **(alloantibodies).**
3. Antigen-antibody complexes are removed by the reticuloendothelial system: spleen, liver, and lymph nodes.

G. Antigen-Antibody Reaction *In Vitro*

1. Reactions are detected by **agglutination or hemolysis.**
2. Some antigen-antibody complexes require two stages for detection: **sensitization** and **lattice** formation.
   a. **Sensitization:** Antibody attaches to antigen but does not produce visible agglutination or hemolysis.
      1) **Factors affecting first stage of agglutination**
         a) **Serum to cell ratio:** This is the amount of antibody compared to the number of cells in solution. Increased amount of serum equals an increase in the number of antibodies in the solution.
         b) **Reaction temperature:** This is the temperature at which the antibody reacts best; most clinically important antibodies react best at **37°C.**
         c) **Incubation time:** This is the time allowed for the antibody to attach to the antigen. This reaction occurs by chance. Times will vary according to the antibody and media used *in vitro* (i.e., albumin, LISS—low-ionic-strength saline).
         d) **pH:** The optimal pH for *in vitro* reactions is 7.
   b. **Lattice formation:** Random collisions of antibody-coated RBCs link antibodies together to form visual agglutination.
      1) **Factors affecting visual agglutination**
         a) **Reaction temperature**
         b) **Incubation time**
         c) **pH**
         d) **Repelling negative charges:** In normal saline, RBCs have a net negative charge that repels other RBCs in solution. This charge inhibits agglutination.
3. **Antigen and antibody agglutination**
   a. **Zone of equivalence:** Antigen and antibody concentrations produce maximum agglutination.
   b. **Prozone (antibody excess):** Too much antibody compared with antigen concentration
   c. **Antigen excess:** Too much antigen compared with antibody concentration
4. **Grading agglutination reactions**
   a. To standardize the strength of agglutination reactions:
      1) **4+ RBC button** is solid with a clear supernatant.

2) **3+ RBC button** breaks into several large clumps, with a clear supernatant.
3) **2+ RBC button** breaks into many medium-sized clumps, with a clear supernatant.
4) **1+ RBC button** breaks into many medium- and small-sized clumps, with background having many free RBCs (appears cloudy).
5) **+^{w} RBC button** breaks into many clumps, barely or not visible macroscopically, with many RBCs in the background (use microscope to see clumps).
6) **0 = no agglutinated RBCs**

5. **Hemolysis** is another indication of antibody-antigen reactions and is caused by **complement activation.** The supernatant appears clear red, with a smaller or nonexistent RBC button.

II. GENETICS

A. Definitions

1. **Chromosomes:** Structures that carry genetic information encoded on double-stranded DNA
2. **Mitosis:** Process of cell division that results in the same number of chromosomes in the new and old cells
3. **Meiosis:** Process of cell division that occurs in gametes resulting in one-half the chromosomes in each new cell
4. **Blood group systems:** Groups of related RBC antigens inherited according to Mendelian genetics
5. **Phenotype:** Physical, observable expression of inherited traits; detectable products
6. **Genotype:** Inherited genes; actual genetic makeup
7. **Pedigree chart:** Visual map that displays a family history and can display inheritance patterns for individual traits
8. **Gene:** Smallest unit of inheritance
9. **Genetic locus:** Site on chromosome where specific genes are located
10. **Alleles:** Alternative forms of a gene
11. **Antithetical:** Opposite form of a gene, different allele
12. **Polymorphic:** Having two or more possible alleles at a locus
13. **Codominant:** Equal expression of both alleles in phenotype
14. **Recessive:** Same allele must be inherited from both parents to be expressed, homozygous
15. **Dominant:** Only one allele must be inherited for it to be expressed; gene product always present
16. **Autosomal:** Genes expressed with equal frequency in males and females, on non-sex chromosome

17. **Sex-linked dominant:** Carried on the X chromosome; no father-to-son transmission; will be expressed if passed from father to daughter or from mother to son
18. **Sex-linked recessive:** It is carried on the X chromosome. Males inherit it from carrier mothers; traits are exhibited most commonly in males (e.g., hemophilia A). Females can exhibit the trait but must inherit it from both carrier mother and affected father.

B. Mendelian Inheritance Principles

1. **Law of Independent Segregation:** Two members of a single gene pair passed from one generation to the next in separate gametes
2. **Law of Independent Assortment:** Traits inherited from different chromosomes expressed separately and discretely
3. **Inheritance patterns:** The inheritance of blood group antigens (A, B, O) can be predicted using a **Punnett square.** Punnett squares have the one person's genotype on the top and the other person's genotype on the side. See Table 5–1■.
4. Each square represents a possible genotype for an offspring. An offspring from these particular parents would have a 25% chance of inheriting any one of the four possible variants. Punnett squares are useful for understanding inheritance of blood groups and ramifications of **heterozygosity** or **homozygosity.**
5. **Homozygous:** Individual inherits **identical alleles** at the same gene locus from both parents.
6. **Heterozygous:** Individual inherits **different alleles** at the same gene locus from each parent.
7. **Dosage effect:** Agglutination reactions are generally stronger for homozygous cells and slightly weaker for heterozygous cells.
8. **Cis:** Genes are inherited on the **same** chromosome.
9. **Trans:** Genes are inherited on **separate** chromosomes. Genes inherited in transposition can weaken the trait's expression.
10. **Linkage and haplotypes**
    a. **Linked genes:** Genes that are close together on a chromosome and inherited as one unit. The Law of Independent Assortment does **not** hold with linked genes.

TABLE 5-1 PUNNETT SQUARE

		Mother's Genotype	
		A	**B**
Father's Genotype	**B**	AB	BB
	O	AO	BO

b. **Haplotype:** Set of genes inherited via one of the two parental gametes
c. **Amorphs:** Genes that do not produce a detectable product

11. **Population genetics:** Statistical calculation to determine the prevalence of antigens in specific populations
    a. **Phenotype calculations:** Determine the frequency of an antigen in a population
    b. If a person has **multiple antibodies,** determine the **percentage of compatible units;** the **frequency for each antibody must be multiplied.** For example, if the individual antigen probabilities in the population are

    30% E-positive, then it is 70% E-negative = 0.70

    78% M-positive, then it is 22% M-negative = 0.22

    80% c-positive, then it is 20% c-negative = 0.20

    Prediction of percentage of compatible units = the product of the individual probabilities or $0.70 \times 0.22 \times 0.20 = 0.03$ or 3% if units are randomly chosen from inventory.
12. **Parentage testing: HLA antigens follow Mendelian genetics** principles and can be used to determine the parents of offspring. HLA genes are polymorphic with many alleles possible at each locus. The more alleles, the less likely it is to find two identical individuals. Parentage testing works on the principle of excluding falsely accused individuals using statistics.

III. ABO AND H BLOOD GROUP SYSTEMS AND SECRETOR STATUS

A. Landsteiner's Rule:

If an individual has the antigen, that individual will not have the antibody. This is a universal law and has few exceptions.

B. ABO Antigens

1. Found on **RBCs, lymphocytes, platelets, tissue cells, bone marrow,** and **organs**
2. These antigens can be secreted by tissue cells if the appropriate genes are present.
3. **Glycolipid** or **glycoprotein**
4. **Developed *in utero*** at 5–6 weeks of gestation
5. **Full expression** of ABO antigens occurs between **2 and 4 years of age.**
6. **Frequencies:** See Table 5–2■.

C. Inheritance and Development of A, B, and H Antigens

1. The **H antigen** is the building block for the **A and B antigens.** There are only two alleles in the *H* gene: *H* and *h*. The ***H* allele is found in 99.99% of the world's population,** and *h* is a rare amorph allele.

TABLE 5-2 FREQUENCIES OF ABO ANTIGENS		
Blood Type	**Whites, Frequency (%)**	**Blacks, Frequency (%)**
O	45	49
A	40	27
B	11	20
AB	4	4

2. The **H antigen** acts as the **acceptor molecule** for the two **sugars** that make up the A and B antigens.
3. The **A blood type** is the H antigen with ***N*-acetylgalactosamine** attached.
4. The **B blood type** is the H antigen with **D-galactose** attached.
5. The **O blood type** is the H antigen with **no additional sugar** attached.

D. ABO Subgroups

1. **Subgroups differ** in the **amount of** the **antigen expressed** on the RBCs. Subgroup A_1 possesses both A and A_1 antigens on the RBC surface. Subgroup A_2 only expresses A antigen.
2. **Blood group A** has two major subgroups, **A_1 and A_2. 80%** of group A people are **A_1,** and **20%** of group A people are **A_2.**
3. People with subgroups of the A antigen can produce antibodies against A_1 antigen.
4. Subgroups of A include **A_1, A_2, A_3, A_x, A_m, A_{el},** and **A_{bantu}.**
5. **Subgroups of A** can be **detected by polyclonal Anti-A,B.** This is produced by Group O individuals only. Anti-A,B will agglutinate A subgroups because it has specificity for both A and B antigens but cannot be separated into Anti-A and Anti-B. Anti-A_1 lectin is active against A_1, but not the other A subgroups.
6. Subgroup **A_3** characteristically produces a mixed-field reaction with polyclonal Anti-A and polyclonal Anti-A,B.
7. If weak subgroups of A in recipients are not detected, there is no harm in a person with the subgroup receiving type O blood. However, if the person with the **weak subgroup of A donates blood** that is **transfused to a group O** patient, **intravascular hemolysis** may result.

E. A and B Are Codominant Traits:

If the **allele** is **present,** the **antigen** will be **expressed.** ***O*** is **an amorph allele** that produces no transferase to add sugars to the H determinant site.

F. Anti-A and Anti-B: These antibodies are produced by humans, who **lack the corresponding antigen,** as a result of exposure to naturally occurring substances that resemble A and B antigens.

G. Anti-A and Anti-B Are IgM Antibodies: This means they **activate complement** and cause **visible RBC agglutination.** They may cause **hemolysis** at room temperature.

H. Routine ABO Grouping

1. **Forward type:** Person's **RBCs** are mixed with reagent Anti-A and Anti-B.
2. **Reverse type:** Person's **serum** is mixed with reagent A_1 and B RBCs.
3. **ABO discrepancies occur when the forward and reverse groupings do not agree.**
   a. **Problems with forward grouping** (extra antigen present, weak antigens) could be caused by acquired B phenotype, polyagglutination, rouleaux, ABO subgroups, transfusion of non-type specific blood, and bone marrow or stem cell transplants.
   b. **Problems with reverse grouping** (unexpected antibodies or weak/missing antibodies) could be seen in individuals with A subgroups with Anti-A_1, cold alloantibodies, cold autoantibodies, and rouleaux, and in a newborn or elderly person.

I. Bombay (O_h) Phenotype

1. Person inherits ***hh*** **genotype.**
2. **Types as an O** (forward and reverse); has **alloanti-H** capable of activating complement and causing a hemolytic transfusion reaction
3. These people can **only** be **transfused with Bombay group blood.** Blood may be collected and frozen as autologous or from siblings who are also Bombay.

J. Secretor Status

1. Two alleles: ***Se*** and ***se***
2. People who inherit ***Se*** are **secretors** and are capable of expressing ABO and *H* antigens in their secretions.
3. A, B, and H antigens, appropriate to the individual's ABO group, are found in saliva, urine, tears, bile, amniotic fluid, breast milk, exudate, and digestive fluids of secretors (*Se*).

IV. Rh BLOOD GROUPS

A. Rh Blood Group System

1. Controlled by two genes *RHD* and *RHCE*. ***RHD*** controls **D expression; no d allele.** ***RHCE*** controls **C, c, E, e expression.**
2. Rh antigens are proteins.

3. **Rh Terminology**
   a. The most common individual antigens are named in the **Fisher-Race** terminology, **D, C, c, E, e, C^w, G, etc.**
   b. **Haplotypes** are often expressed in a modified **Wiener** terminology such as **R_1R_1 for CDe/CDe.**
4. **Phenotype:** RBC antigens identified with specific antisera; **Genotype:** Genes present on person's chromosomes
5. **Rh system antigens**
   a. **D antigen: Most immunogenic** of Rh antigens
   b. **Weak D**
      1) Weak D occurs when **D** is **weakly expressed. Weak D** must be **detected** by an **IAT** (indirect antiglobulin test).
      2) **Genetic cause:** Weaker expression of the ***cDe*** **haplotype** may fail to react by direct agglutination testing, but it will **react strongly** by the **IAT.**
      3) **Position effect:** Occurs when the **C antigen** is inherited **trans** to the **D antigen. This weak D** may be detected **without** carrying the test to the antiglobulin phase.
      4) **Partial D:** Occurs when only **part** of the **D antigen** is inherited. There are multiple epitopes that make up the D antigen. A partial-D individual **lacks one or more** of these **epitopes** and is capable of **making antibody** to the epitopes that s(he) lacks. Partial-D individuals are usually detected because the antigen reacts strongly with monoclonal reagents. A partial D is suspected when a seemingly D-positive person makes anti-D after transfusion with D-positive blood.
      5) **Weakly reactive D** means a person **is D-positive.** AABB *Standards* state that all **Rh-negative donor units** must be **tested for weak D,** and those units that test **positive** must be **identified as D-positive.** However, **weak-D recipients** are **transfused with D-negative** blood.
   c. **Other Rh system antigens**
      1) **f or ce:** If *c* and *e* are present on the same haplotype, f antigen is expressed.
      2) **Ce or rh_i:** *C* and *e* are inherited as a haplotype made by D-positive individuals who make anti-C.
      3) **C^w:** Low-frequency antigen
      4) **V or ce^s:** 30% prevalence in African-Americans
      5) **G:** In test tube appears to be anti-D and anti-C
      6) **Rh:29:** Antibody to Rh:29 is the antibody to the high-frequency Rh antigen made by Rh_{null} people.
   d. **Unusual phenotypes**
      1) **D deletion:** No reaction occurs when tested with anti-E, anti-e, anti-C, and anti-c. **Written as D – –.**
      2) **Rh_{null} phenotype:** This appears to have no Rh antigens. The membranes of their RBCs are abnormal and the RBCs have a shortened

life span. This can result from inheriting two nonfunctional *RHCE* alleles along with the dual deletion of the *RHD* alleles. Rh_{null} phenotype can also result from inheriting two recessive regulator alleles at the *RHAG* locus. The latter individuals pass on normal *RHD* and *RHCE* alleles to their children.

6. **Rh antibodies**
   a. Produced in humans through **pregnancy or transfusions**
   b. **IgG antibody;** Rh antibodies generally do not activate complement
   c. Optimal reaction temperature: **37°C**
   d. Reaction phase: **AHG** (antihuman globulin)
   e. **Agglutination enhancement** occurs with LISS, enzymes, and PEG (polyethylene glycol).
   f. Stronger reactivity of antibody with cells from homozygous individuals is shown with anti-C, anti-c, anti-E, and anti-e (dosage).
   g. C and e and E and c are usually found together.
   h. These antibodies produce **hemolytic transfusion reactions (HTRs).** Antibodies may not be currently detectable, but the person should always receive **antigen negative blood** if they have a history of Rh antibodies.
   i. Rh antibodies can cause **hemolytic disease of the newborn (HDN),** because they can cross the placenta. **Rh immune globulin** (RhIG) administered after delivery (within 72 hours) can **protect** a woman **from making anti-D.**

V. OTHER BLOOD GROUP SYSTEMS

A. Kell Blood Group System

1. Abbreviation: **K**
2. Antibody class: **IgG**
3. Optimal reaction temperature: **37°C**
4. Reaction phase: **AHG**
5. Enzyme treatment: **No effect**
6. **Antigens:** K (Kell), k (Cellano), Kp^a, Kp^b, Kp^c, Js^a, Js^b, and Ku; **common Kell** system antigens **k, Kp^b, and Js^b**
7. **Allelic pairs:** Include K and k, Kp^a and Kp^b, Js^a and Js^b
8. **K is very immunogenic.** Although the K antigen is found in only about 9% of the population, **anti-K** is encountered quite frequently and can **cause HTR** and **HDN.**
9. **$Kell_{null}$:** This is also known as **K_0.** It occurs when **RBCs lack** the **Kell antigens** but **have** the **Kx antigen.**
10. The **Kx antigen** is produced by a gene located on a **different chromosome** than the Kell system genes. This antigen is inherited independently from the Kell antigens; the Kx antigen structure appears to be required for the expression of the Kell system antigens. **K_{null}** individuals have **increased** amounts of **Kx.**

11. **McLeod phenotype**
    a. Individuals who have an **alteration** of the **allele-producing *Kx*** on the X chromosome **lack Kx** on the red blood cells and have greatly **decreased expression of Kell antigens.**
    b. These individuals have decreased RBC survival as well as RBC morphologic and functional abnormalities.

B. Duffy Blood Group System

1. Abbreviation: **Fy**
2. Antibody class: **IgG**
3. Optimal reaction temperature: **37°C**
4. Reaction phase: **AHG**
5. Enzyme treatment: **Destroys Fy^a and Fy^b**
6. Clinically significant:
    a. **Anti-Fy^a** and **anti-Fy^b** can **cause HTR** and **HDN.**
    b. The Fy(a–b–) phenotype is more resistant to malarial infection by *Plasmodium vivax.*
7. **Antigens:** Fy^a, Fy^b
8. **Four phenotypes:** Fy($a+b-$); Fy($a-b+$); Fy($a+b+$); Fy(a–b–)
9. **Alleles:** Fy^a, Fy^b, and *Fy* (silent allele)
10. Commonly show dosage effect: Weak antibodies react more strongly with homozygous cells.

C. Kidd Blood Group System

1. Abbreviation: **Jk**
2. Antibody class: **IgG**
3. Optimal reaction temperature: **37°C**
4. Reaction phase: **AHG**
5. Enzyme treatment: **Enhances agglutination**
6. Clinically significant: Associated with **HTR** and **mild HDN**
7. **Antigens:** Jk^a, Jk^b, Jk3
8. **Four phenotypes:** Jk($a+b-$); Jk($a-b+$); Jk($a+b+$); Jk(a–b–)
9. **Alleles:** ***Jk^a*** codes for Jk^a and Jk3; ***Jk^b*** codes for Jk^b and Jk3.
10. **Show dosage effect:** Weak antibodies agglutinate homozygous cells more strongly than heterozygous cells.
11. These antibodies **bind complement.**
12. These **antibodies deteriorate in storage,** declining quickly to below the detectable level in human serum, and commonly **cause delayed HTR** (DHTR).

D. Lutheran Blood Group System

1. Abbreviation: **Lu**
2. Antibody class: **Lu^a IgM; Lu^b IgG**
3. Optimal reaction temperature: **Lu^a 4°C; Lu^b 37°C**

4. Reaction phase: **Lu^a room temperature; Lu^b AHG**
5. Enzyme treatment: **Variable effect**
6. Clinically significant:
   a. No clinical significance. **Anti-Lu^a** can be present without prior transfusion or pregnancy.
   b. **Anti-Lu^b** is rare and associated with HTR and HDN.
7. **Antigens:** 18 total, including Au^a and Au^b
8. **Alleles:** *Lu^a*, *Lu^b*

E. Lewis Blood Group System

1. Abbreviation: **Le**
2. Antibody class: **IgM**
3. Optimal reaction temperature: **Most often 4°C, sometimes 37°C**
4. Reaction phase: **Room temperature, 37°C,** and **AHG**
5. Enzyme treatment: **Enhanced agglutination**
6. Clinically significant: **No**
7. Produced by tissue cells and secreted into fluids. The antigens are adsorbed onto the RBC membranes.
8. May take 6 years to fully develop these antigens.
9. **Genetics: If *Le* gene inherited, Le^a is adsorbed onto RBCs—Le(a+b–). Le^a** is the only antigen that can be **secreted** by a **nonsecretor.**
10. **If *Se* gene is also inherited, Le^b is adsorbed onto the RBC—Le(a – b +).**
11. Bombay phenotypes are Le^a positive if they inherit the *Le* gene.
12. Cells type as Le(*a*+*b*+) (transiently during first years of life), Le(*a*+*b*−), Le(*a*−*b*+), Le(a–b–).
13. Lewis antibodies are sometimes formed during pregnancy but weaken and disappear after delivery.

F. I Blood Group System

1. Abbreviation: **I**
2. Antibody class: **IgM**
3. Optimal reaction temperature: **4°C**
4. Reaction phase: **Immediate spin (IS)** and **occasionally 37°C**
5. Enzyme treatment: **Enhanced agglutination**
6. Clinically significant: **No**
7. It can be a bothersome antibody, masking the reactions of a clinically significant alloantibody. May need to prewarm cell suspension and reagent or do cold autoabsorption to find clinically significant alloantibodies.
8. Strong anti-I is associated with *Mycoplasma pneumoniae* infection.

G. P Blood Group System

1. Abbreviation: **P_1**
2. Antibody class: **IgM (anti-P_1)**

3. Optimal reaction temperature: **4°C**
4. Reaction phase: **IS, 37°C** and **AHG**
5. Enzyme treatment: **Enhanced agglutination**
6. Clinically significant:
   a. Anti-P_1 is not clinically significant.
   b. **Anti-P_1 + P + P^k** is an **IgG clinically significant** antibody.
7. **Phenotypes:** P_1, P_2, p, P_1^k, P_2^k, and Luke
8. **Alleles:** P_1, P, P^k, and p
9. Anti-P_1 can be neutralized by soluble P_1 reagent.
10. **Autoanti-P** is **Donath-Landsteiner antibody.** Naturally occurring biphasic antibody associated with paroxysmal cold hemoglobinuria. It binds to the antigen on the patient's RBCs in the cold and fixes complement. The RBCs are hemolyzed when the temperature reaches 37°C.
11. Patients with **autoanti-P** may require a **blood warmer for transfusion.**
12. Anti-PP_1P^k is found in individuals of the p phenotype. It is clinically significant and associated with spontaneous abortions. Need compatible blood from other p phenotype individuals.

H. MNS Blood Group System

1. **M and N antigens**
   a. Abbreviation: **MN**
   b. Antibody class: **IgM**
   c. Optimal reaction temperature: **4°C** or **37°C**
   d. Reaction phase: **IS, 37°C,** or **AHG**
   e. Enzyme treatment: **Destroys antigens**
   f. Clinically significant: **No**
   g. **Antigens:** M and N associated with **glycophorin A**
2. **S and s antigens**
   a. Abbreviation: **Ss**
   b. Antibody class: **IgG**
   c. Optimal reaction temperature: **37°C**
   d. Reaction phase: **AHG**
   e. Enzyme treatment: **Variable effect**
   f. Clinically significant: **Yes**
   g. **Antigens:** S, s, and U associated with **glycophorin B**
3. Anti-M
   a. It is **clinically significant** if **IgG; IgM** antibody is **not** clinically significant.
   b. Demonstrates dosage effect
4. Anti-N is very rare.
5. Anti-S, Anti-s, and Anti-U
   a. **Clinically significant,** causing **HTR** and **HDN**
   b. Anti-U is rare and occurs in S–s–U– people.

I. Miscellaneous Blood Group Systems

1. **Diego:** Di^a, Di^b, Wr^a, Wr^b; Di^b and Wr^b are high-incidence antigens.
2. **Cartwright:** Yt^a and Yt^b; Yt^a is a high-incidence antigen.
3. **XG:** Xg^a antigen has a higher incidence in females than in males.
4. **Scianna:** Sc1, Sc2, and Sc3; Sc1 and Sc3 are high-incidence antigens.
5. **Dombrock:** Do^a, Do^b, Gy^a, Hy, and Jo^a; Gy^a, Hy, and Jo^a are high-incidence antigens.
6. **Colton:** Co^a, Co^b, and Co3; Co^a is a high-incidence antigen.
7. **Chido/Rodgers:** Ch^a and Rg^a are both high-incidence antigens.
8. **Gerbich:** Ge2, Ge3, and Ge4 are high-incidence antigens.
9. **Cromer:** Cr^a and several others are high-incidence antigens.
10. **Knops:** Kn^a, McC^a, Sl1, and Yk^a are high-incidence antigens.
11. **Cost:** Cs^a and Cs^b; Cs^a is a high-incidence antigen.
12. **Vel:** Vel is a high-incidence antigen. Anti-Vel is a hemolytic, clinically significant antibody.
13. **John Milton Hagen:** JMH is a high-incidence antigen.
14. **Sid:** Sd^a is a high-incidence antigen.

VI. BLOOD BANK REAGENTS AND METHODS

A. Principle of Blood Bank Tests

Ag + Ab $\leftrightarrow$ Ag-Ab reaction

B. Routine Blood Bank Testing Procedures

1. **ABO/Rh typing**
   a. Detects **A, B,** and **D antigens**
   b. **Source of antigens:** Patient's RBCs (forward grouping/typing); reagent RBCs (reverse grouping)
   c. **Source of antibodies:** Reagent anti-A, anti-B, and anti-D (forward grouping/typing); patient's serum (reverse grouping)
2. **Antibody screen**
   a. Detects specific **antibodies to RBC antigens**
   b. **Source of antigens:** Reagent antibody screening cells
   c. **Source of antibodies:** Patient's serum
3. **Antibody identification**
   a. Identifies antibodies to RBC antigens
   b. **Source of antigens:** Reagent antibody panel cells (10–16 cells)
   c. **Source of antibodies:** Patient's serum
4. **Crossmatch**
   a. Determines **compatibility** of **donor RBCs** with **recipient's blood**
   b. **Source of antigens:** Donor cells
   c. **Source of antibodies:** Recipient's serum

C. Types of Blood Bank Reagents

1. **Reagent RBCs** possess **known antigens** and are treated to prolong their life span.
2. **Antisera** contain known **antibodies** against specific RBC antigens.
3. **Antiglobulin reagents** contain poly- or monospecific **antibodies** against human antibodies.
4. **Potentiators** are solutions that **enhance** the **formation of antigen-antibody complexes.**

D. Regulation of Reagent Production

1. Blood bank reagents are licensed by the Center for Biologics Evaluation and Research of the Food and Drug Administration (FDA).
2. FDA specifies potency and specificity of reagents before production.

E. Reagent Antisera

1. **Polyclonal: Many B cell clones** produce antibodies against antigens.
2. **Monoclonal: A single B cell clone** produces antibody against an antigen.
   a. **Advantages:** Endless production, exactly the same reagent in each batch, no human/animal sources, no contamination
   b. **Disadvantages:** Single specificity; may not react with all portions of RBC antigen
3. **Blended monoclonal:** Reduces disadvantages of single clone
4. **ABO antisera**
   a. **Anti-A** and **Anti-B reagents** are used to determine if patient is A, B, AB, or O. See Table 5–3■.
   b. **Anti-A reagent** is colored with a **blue dye; Anti-B reagent** is colored with a **yellow dye.**
   c. Suspension of **patient's cells** is added to antisera.
   d. Agglutination read at immediate spin. These antibodies are **IgM** and react best at room temperature or 4°C.

TABLE 5-3 ABO ANTISERA REACTIONS

	Anti-A Reagent	Anti-B Reagent
Type A	+	0
Type B	0	+
Type AB	+	+
Type O	0	0

5. **D typing**
   a. These are **important antigens** to detect because **antibody-antigen reactions *in vivo* cause HTR and HDN.**
   b. Two types of reagents:
      1) **High protein:** Older reagent; need to run an Rh control with this reagent because the protein in the diluent may cause false positive reactions in patients with autoantibodies or abnormal serum proteins.
      2) **Low-protein monoclonal:** Rh control is usually not required. Only need to perform a control when patient has abnormal serum proteins.
6. **Antiglobulin reagents**
   a. **Polyspecific:** Detects both anti-IgG and anti-C3; used often in direct antiglobulin tests (DAT)
   b. **Monoclonal:** This may be used to differentiate between antibodies to IgG and C3. Monospecific (antihuman IgG) reagents are typically employed for tests requiring an antiglobulin phase of testing.
7. **Reagent RBCs**
   a. **IgG-coated control cells (Check cells):** *AABB Standards for Blood Banks and Transfusion Services* require a control to **ensure antiglobulin reagent reactivity in each negative antiglobulin test tube.** Check cells are prepared by attaching an IgG antibody to RBCs (sensitized RBCs).
   b. **A_1 and B cells for reverse grouping:** These are used to **confirm front typing** results. These cells **detect ABO antibodies.** Landsteiner's Law: If the **patient's RBCs have an antigen,** they do **not** have the antibody in the serum.
   c. **Antibody screening cells:** These are used to **detect antibodies present in a patient's serum.** Antibodies must be detected before patients are transfused to prevent hemolytic transfusion reactions and/or death. Each set of screening cells has two or three antigenically different RBC reagent red cells. The **antigens of each cell are known** and printed on an **antigram** included with each set.
   d. **Antibody panel cells:** Antibody identification procedures use **panel of RBCs** whose **antigens** are **known.** The panel consists of 10 to 20 vials of these RBCs. Every panel has an antigenic profile that lists all of the known antigens on each vial of RBCs.
   e. **Other methods for antigen-antibody reaction detection**
      1) **Gel technology:** This technique uses **dextran acrylamide gel** combined with reagents or diluent. Anti-IgG cards are used for DATs and IATs.
      2) **Microplate methods:** The traditional tube method is adapted to the microtiter plate, in which **smaller volumes of serum and cells are used,** and it is read on an automated photometric instrument. The cell buttons are resuspended by tapping the sides of the plate.
      3) **Solid-phase adherence methods**
         a) **RBC screening cells** are **bound to surface** of microtiter plates.
         b) Add patient's serum.

c) RBCs capture IgGs.
d) Plates washed
e) Indicator cells (anti-IgG-coated RBCs) are in contact with bound antibody.
f) **Negative** = RBC button; **Positive** = RBCs (indicator cells) on sides and bottom of wells

4) **Indirect antiglobulin test (IAT)**
a) Purpose of the **IAT** is to **detect *in vitro* sensitization of RBCs.**
b) In this procedure, reagent red cells are mixed with patient's serum, then incubated at 37°C to allow IgG antibodies to attach to the RBCs. The solution is then washed to remove unbound proteins. **AHG is added to detect *in vitro* sensitization of RBCs.**
c) **False positive tests** result from RBCs being agglutinated before the washing step (cold agglutinin), improper RBC suspension, dirty glassware, and overcentrifugation.
d) **False negative tests** result from poor washing of RBCs, testing being delayed, loss of reagent activity, no AHG added, or use of an improper RBC suspension.

5) **Potentiating media (antibody enhancers)**
a) **Definition:** Reagents added to the *in vitro* antiglobulin test to **enhance antigen-antibody complex formation**
b) **LISS** increases antibody uptake of antigen.
c) **Bovine albumin** (22% or 30%) allows sensitized cells to come close together to form agglutination lattices.
d) **Polyethylene glycol (PEG) additive** concentrates antibodies and creates a low-ionic solution to allow greater antibody uptake.
e) **Proteolytic enzymes: Papain, ficin,** and **bromelin** are used. **Enzymes remove certain structures** from the RBC and **enhance** the **access of antibodies** to other less superficial structures on the RBC. Antibodies that are **enhanced** include **Rh, Kidd,** and **Le** blood group systems. The following **antigens** are **destroyed** by the enzymes: **M, N, S, Xg^a, Fy^a,** and **Fy^b.**

VII. DIRECT ANTIGLOBULIN TESTING

A. Direct Antiglobulin Test (DAT)

1. RBCs may combine with antibodies without agglutinating.
2. **Antihuman globulin (AHG)** is an *in vitro* reagent used to **agglutinate RBCs with antibodies attached** to them (sensitized RBCs).
3. Direct antiglobulin test
   a. Ordered to **detect IgG and/or complement proteins attached to RBCs** in autoimmune hemolytic anemia, hemolytic disease of the newborn, a drug-related mechanism, or a transfusion reaction

b. Indicates **immune-mediated** *in vivo* RBC destruction (antibodies attached to RBCs ***in vivo***)
c. **Procedure**
   1) Patient's RBCs are washed three times with normal saline to remove unbound proteins.
   2) Polyspecific AHG is added after washing.
   3) Agglutination indicates that the patient has antibodies or complement proteins attached to RBCs.
d. **Specimen of choice:** EDTA negates the *in vitro* activation of complement.

VIII. IDENTIFICATION OF UNEXPECTED ALLOANTIBODIES

A. Detection of Atypical and Unexpected Antibodies

1. Antibodies other than ABO in a person's blood
2. **Antibody screen**
   a. **Purpose:** To detect antibodies in patients requiring transfusions, pregnant women, blood and blood product donors, and patients with suspected transfusion reactions
   b. **Screening cells:** Two to three different group O cells with known antigens included in an antigram
   c. **Procedure**
      1) Mix known RBCs with patient's serum.
      2) Add potentiator and incubate at 37°C.
      3) Spin and read results (if applicable to potentiator).
      4) Wash three times with saline.
      5) Add AHG, spin, then read results.
      6) Read all negative results macroscopically (some facilities read all negative results microscopically).
      7) Add IgG-coated control cells (check cells) to all tubes with a negative reaction at AHG. Check cells must be agglutinated or the test must be repeated.
      8) Spin and read for agglutination.
   d. **Results:** Any **agglutination** at any phase of testing indicates an atypical or unexpected antibody.
   e. **Autocontrol:** Use patient's serum and patient's RBCs. Autocontrol is used to detect **autoantibodies.** Often performed in conjunction with the antibody screen and is tested in all phases.
   f. **Potentiators** are used to **enhance antibody detection.**
   g. **Patient history:** A patient's transfusion and antibody history should be researched at that institution before transfusing the patient.
3. **Antibody identification**
   a. **Antibody panel:** Type **O cells with known antigens;** usually 10–20 bottles of different cells with known antigens
   b. **Purpose:** To **identify alloantibodies** detected in patient's serum

4. **Panel interpretation**
   a. **Autocontrol** determines if antibody is autoantibody or alloantibody.
   b. **Phases:** The **reaction phase** of the antibody is **important.** It will determine if the antibody is IgG or IgM. **Room temperature** reactions usually indicate an **IgM** antibody. Reactions at 37°C and/or AHG usually indicate an IgG antibody.
   c. **Reaction strength**
      1) **Single-strength** reactions usually indicate a **single antibody.**
      2) **Various-strength** reactions usually indicate **multiple antibodies or dosage.**
   d. **Ruling out**
      1) **Negative reaction (0)** indicates that the antibody(ies) does(do) not react with any antigen on that RBC. However, if that cell is heterozygous for an antigen, antibody may be showing dosage.
      2) **Positive reaction** (+) should **never** be used at any phase of testing to rule out! Always **use** this in **identification.**
   e. Determining the **antibody specificity**
      1) **Single antibody:** If there is only one antibody, the reactions will match the antigen pattern on the antigram.
      2) **Multiple antibodies:** If there is more than one antibody, the reactions are difficult to match with a single antigen pattern on the antigram. Multiple antibodies may react with varying strengths.
   f. **Rule of Three**
      1) Are there **three cells** with **positive reactions** from the panel cells?
      2) Are there **three cells** with **negative reactions** from the panel cells?
      3) If the answers to both of the two previous questions are yes, then there is a **95% probability** that the **antibody is correctly identified.**
   g. **Phenotype patient**
      1) This is required to confirm antibody identification. If the patient is negative for the antigen, an antibody is possible.
      2) In the patient who has not been recently transfused, when his(her) RBCs are positive for the antigen, the antibody to that antigen is usually not produced.
5. **Multiple antibody resolution**
   a. May need to perform more tests to identify antibodies.
   b. Selected cells can be used to complete identification; use rule of three. Individual cells from a second panel can be used.
   c. Additional technique
      1) **One-stage enzyme:** Incubate patient's serum, enzyme, and RBCs.
      2) **Two-stage enzyme:** Pretreat panel or screening cells with enzymes, wash, and perform IAT without additional enhancements.

B. Antibodies to High-Frequency Antigens

1. **Definition:** Antibodies produced against antigens that occur in at least 98% of the population
2. When interpreting panels, you know you have an antibody to a high-frequency antigen when
   a. The autocontrol is negative.
   b. Reactions occur with most or all panel cells at AHG.
   c. Reaction strength in all panel cells is the same.
3. **Additional testing:** Under the **rule of three,** there must be at least three positive and three negative cells. Choose cells from other panels that lack various high-incidence antigens.
4. **Clues for identification of antibodies to high-frequency antigens**
   a. I, H, P, P_1, and $P+P_1+P^k$ produce **room temperature** reactions.
   b. Lu^b, Ch, Rg, Cs^a, Kn^a, McC^a, Sl^a, and JMH produce weak and varying reactions at **AHG.**

C. Antibodies to Low-Frequency Antigens

1. If the **antibody screen** is **negative** and the **crossmatch** is **positive,** suspect antibodies to low-frequency antigens.
2. **Low-frequency antigens** include Lu^a, C^w, Kp^a, Wr^a, V, Bg^a, VS, and Co^b.
3. Crossmatching further units will usually result in compatibility.
4. If the antibody is found in a pregnant woman, test the father's RBCs with the mother's serum to determine if the fetus is in danger of hemolytic disease of the newborn.

D. Enhancing Weak IgG Antibodies

1. If weak reactions are encountered that do not fit the pattern of a known antigen, repeat panel using different potentiators, increase incubation time, and/or increase serum to cell ratio.

E. Alloantibodies

1. Cold antibodies that react at 4°C and/or room temperature are usually not clinically significant. These antibodies can hide a clinically significant alloantibody.
2. Prewarmed techniques or adsorption of cold antibody can help detect any alloantibodies present. If the cold antibody reacts at 37°C, it may be clinically significant.

F. Autoantibodies

1. Can be detected by a **positive DAT** or **positive autocontrol**
2. Can be produced in response to drug effects, cold autoimmune disease, pneumonia, warm autoimmune disease, infectious mononucleosis, etc.

G. Cold Panels

1. Cold panels are done to **identify "cold" antibodies.**
2. **Antibody panels** are **performed** with the incubation at **4°C** instead of 37°C.
3. Because **most cold autoantibodies** are either **anti-I, anti-H, or anti-IH,** an abbreviated or "mini" cold panel can be performed.
   a. Select cells for panel: Use screening cells (type O), an autocontrol, cord blood, or i-positive cells from a commercial panel, and type specific cells for the patient (e.g., A cells for type A, B cells for type B).
   b. Add two drops of patient's serum to cells and incubate at 4°C for 20 minutes.
   c. Centrifuge, resuspend, and grade reactions after incubation.
   d. Interpretation: See Table 5–4■.

H. Avoiding Cold Antibodies

1. Use IgG antihuman globulin.
2. Skip the immediate spin or room temperature phase.
3. Use 22% albumin instead of LISS.
4. **Use prewarmed technique.**
   a. Using 2–5% cell suspensions, place one drop of each panel cell and one drop of patient's cells into their respectively labeled tubes and incubate at 37°C for 10 minutes.
   b. Simultaneously, warm patient's serum at 37°C for 10 minutes.
   c. Add prewarmed serum to prewarmed panel cells and incubate at 37°C for 30 minutes.
   d. Wash three times in saline prewarmed to 37°C.
   e. Add AHG, spin, read, and grade reactions.
   f. Interpret reactions.
   g. Add IgG-coated control cells to negative tubes.

I. Adsorption Techniques

1. If patient was not transfused in last 3 months, an autoadsorption can be performed.
2. If patient was transfused within the past 3 months, use allogeneic cells lacking the same antigens as the patient for adsorption.

TABLE 5-4 INTERPRETATION OF ANTIBODY PANEL TO IDENTIFY COLD ANTIBODIES

	Screening Cell I	Screening Cell II	Autocontrol	Group O Cord	Type Specific
Anti-I	3+	3+	3+	0	4+
Anti-IH	3+	3+	3+	1+	2+
Anti-H	3+	3+	2+	2+	2+

3. **Cold autoadsorption**
   a. Incubate patient's serum and cells at 4°C for 30–60 minutes.
   b. Remove serum and use serum for panel to test for alloantibody.
4. **Warm autoadsorption**
   a. Incubate patient's serum and cells at 37°C for 30–60 minutes.
   b. Remove serum and use serum in panel to test for alloantibody.

J. Elution

1. IgG that attaches to RBCs *in vivo* can be removed by elution (*in vitro*).
2. **Three types of elution techniques**
   a. **Intact RBC antibody removal** uses buffers to remove the antibody from the RBC without destroying the RBC.
   b. **Digitonin** releases the antibody by destroying the RBCs.
   c. **Lui freeze-thaw** is used to **remove IgM antibodies** (usually A or B) present on newborn RBCs.
3. Once antibody releases, the last wash and the eluate supernatant are tested on a panel.
4. The last wash panel should be negative, and the eluate supernatant should reveal alloantibodies.

IX. PRETRANSFUSION TESTING

A. Compatibility Testing

1. Entails recipient identification, specimen collection and handling, review of patient's blood bank records, ABO/Rh typing, antibody screen, antibody identification, ABO/Rh confirmation on donor units, crossmatching, screening donor units for antigens for which a recipient has an antibody(ies) in his(her) serum, and the actual transfusion.
2. **Definitions**
   a. **Full (complete) crossmatch:** Testing donor cells with recipient serum; carried through all phases and check cells
   b. **Compatible crossmatch:** No agglutination or hemolysis at any phase of testing performed
   c. **Incompatible crossmatch:** Agglutination or hemolysis at any phase of testing performed
   d. **Immediate-spin crossmatch:** Performed only at room temperature; IS is done with donor cells and recipient serum. It is designed to **detect ABO incompatibility.** It is appropriate to perform IS, if recipient's current and historical antibody screening is negative.
   e. **Electronic crossmatch:** Recipient ABO/Rh is tested in duplicate and results are entered into a validated blood bank computer system. The recipient's transfusion history is researched through the computer. If the recipient has not been transfused in the last 3 months and his(her)

antibody screen, both current and historical, is negative, blood for the recipient is issued without any additional testing.

3. **Purpose of crossmatch**
   a. To prevent transfusion of incompatible red blood cells
   b. To maximize RBC life after transfusion
4. **Limitation of crossmatches**
   a. Does not guarantee normal RBC cell survival *in vivo*
   b. Does not detect transfusion-transmitted bacteria, viruses, or parasites
   c. Does not detect allergic reactions
   d. Does not detect WBC antigens
   e. Will not prevent antibody production to foreign antigens present on donor's RBCs
   f. Does not prevent delayed transfusion reactions
5. **Procedure**
   a. Blood sample: Acceptable blood collection tubes are plain red top, yellow top (acid citrate dextrose or ACD, Formula B), purple top (EDTA), and blue top (citrate).
      1) Hemolyzed specimens are not acceptable.
      2) Specimens must be no older than 72 hours for patients transfused or pregnant within the last 3 months.
      3) AABB *Standards* state that the following information must be on the tube:
         a) Patient first and last name; must match name on armband
         b) Unique identifying number on patient sample and requisition
         c) Date of collection
         d) Signature or initials of phlebotomist
      4) The patient must be able to state his(her) name and/or be identified by name band on arm before the tube can be drawn. Information on the tube must match information on the requisition.
6. **AABB *Standards*** require **comparison** of **current** blood bank **workup with other** blood bank **tests performed** on the same patient **within 12 months** (including blood type, typing problems, allo- or autoantibodies, transfusion reactions, or special requirements).
7. AABB *Standards* states that the **ABO** must be **repeated** on all units received into the blood bank, and **Rh** must be **tested** on all **Rh-negative** units.
8. **AABB *Standards* for crossmatches**
   a. Use recipient serum and donor cells (segment taken from the bag of the unit to be transfused).
   b. **Immediate-spin crossmatch:** This is used when recipient has no history of alloantibodies and current antibody screen is negative. The donor cells and recipient serum or plasma are added to a tube. This tube is spun and the reaction is graded. If **negative,** the recipient is transfused with this unit of blood. If **positive,** the crossmatch must be carried out as an antiglobulin crossmatch: IS, 37°C incubation, and AHG phases.

c. **Antiglobulin crossmatch:** This is performed when a history of an alloantibody or the detection of one in the current antibody screen warrants an antiglobulin crossmatch. **AHG crossmatch** involves IS phase, addition of potentiator, 37°C incubation phase, three washes, antiglobulin phase, and finally IgG-coated control cells.
d. **Electronic crossmatch:** AABB *Standards* require
   1) Validated computer system
   2) Validated studies submitted to FDA
   3) Two identical ABO typings on recipient
   4) ABO on current sample
   5) ABO typing by two laboratorians or on two different samples
   6) Computer has donor unit information: Product name, ABO and Rh, unique number, and interpretation of ABO confirmation test.
   7) Computer system contains recipient ABO and Rh.
   8) Computer alert for ABO incompatibilities
   9) Method to verify the correct entry of all data
   10) Advantages: Increased time efficiency, decreased sample volume requirement for patients needing numerous crossmatches
   11) Better inventory management

9. Tagging, inspecting, and issuing blood products
   a. **Every unit to be transfused is tagged.** The tag must contain the patient's full name, unique identification numbers, name of product, donor number, expiration date, ABO and Rh of unit, crossmatch interpretation, and identification of person doing testing or selecting unit.
   b. **Inspecting the unit:** Each unit must be inspected for expiration date, ABO and Rh, discoloration, clots, and bacterial contamination before release for transfusion.
   c. **Issuing:** Person taking the unit must have a request form that has the patient's full name, unique number, and product needed. Both persons issuing and receiving the unit must record their initials or sign that they have checked the unit tag against the request form and record the date and time. If RBCs are not stored in a monitored refrigerator or transfused within 30 minutes of issue, the unit must be returned to the blood bank.
10. **Incompatible crossmatches**
   a. Causes for an **incompatible immediate-spin crossmatch:** Wrong patient identification, wrong sample identification, cold alloantibody, presence of anti-A_1, or a cold autoantibody
   b. Causes for an **incompatible AHG crossmatch:** Alloantibody or autoantibody in patient's serum
11. **Emergency release of uncrossmatched blood**
   a. **Emergency release** must be **signed by physician** requesting blood.
   b. Unit must be tagged just like when performing a crossmatch. **Note that the blood** is an emergency release and **not crossmatched.**

c. Must have full patient name and unique identification number, donor unit number, ABO and Rh, and expiration date on tag, requisition, and blood bank records.
d. **Segments** are **removed** from the unit before issuing so the blood can be **crossmatched after the release of the unit.**
e. Name of the person issuing the unit must be on the requisition and blood bank records.

12. **Massive transfusion**
    a. **Definition:** Total blood volume replacement within 24 hours (approximately 10–12 units)
    b. Each facility has a policy on when a new recipient sample is needed and if crossmatching is necessary.
13. **Maximum surgical blood order schedule (MSBOS):** Procedures are performed according to the surgery a patient is having. Choices include type and screen, crossmatch for two units, crossmatch for four units, or crossmatch for six units.
14. **Crossmatching autologous units**
    a. Blood is **pre-donated by recipient for** use during or after **recipient's own surgery.**
    b. The blood must be transfused to intended patient; it **cannot be given to anyone else.**
    c. Testing for infectious diseases is not required.
    d. **Immediate-spin crossmatch** is performed before issuing blood for transfusion.
15. **Crossmatching infants less than 4 months old**
    a. Newborns develop antibodies by 4–6 months of age. At less than 4 months of age, any antibodies in the reverse grouping or antibody screen are of maternal origin. **Pretransfusion testing** is only **ABO** (forward grouping) and **Rh.**
    b. If alloantibodies are detected in the mother's or infant's serum, the infant is transfused with units negative for the corresponding antigens.
16. **Pretransfusion testing** for fresh-frozen plasma (FFP), platelets, cryoprecipitate, plateletpheresis, and granulocyte concentrates is **ABO grouping.** These products are preferably ABO group specific or compatible.

X. HEMOLYTIC DISEASES OF THE NEWBORN

A. Etiology

1. In hemolytic disease of the newborn or erythroblastosis fetalis, **maternal IgG antibodies cross the placenta** and **destroy the baby's RBCs,** which demonstrate the antigen specific for that antibody. Hemoglobin from lysed RBCs is metabolized into unconjugated bilirubin. The mother metabolizes the bilirubin with no problems. The fetus becomes anemic as RBC destruction continues. Cardiac failure and/or hydrops fetalis may result from anemia.

After birth, bilirubin that was previously metabolized by the mother now accumulates in the baby's circulation. The infant is unable to metabolize and excrete the bilirubin because its liver is not functioning at full capacity. The buildup of bilirubin leads to jaundice and can cause deafness, mental retardation, **kernicterus** (bilirubin accumulation causes brain damage), or death.

B. Rh Hemolytic Disease of the Newborn

1. Most severe
2. D-negative mother develops antibodies during first pregnancy with D-positive baby or after transfusion with D-positive RBCs. The mother's anti-D antibodies attack the fetus of subsequent pregnancies if baby's RBCs are D-positive.
3. **Laboratory results on newborn:** Positive **DAT,** increased **serum bilirubin**
4. Exchange transfusion may be needed to avoid kernicterus.
5. **Rh immune globulin** (RhIG) administered to the mother **provides passive anti-D** to prevent an Rh-negative woman from making anti-D. The passive antibodies attach to Rh-positive fetal RBCs that may enter the maternal circulation before the mother's immune system recognizes the fetal D antigen as foreign. The mother is **not alloimmunized** and does not produce her own antibodies against D.

C. ABO Hemolytic Disease

1. Most common form of HDN; A or B babies born to O mother; usually mild disease
2. Usually not treated by transfusion
3. Infants are treated by **phototherapy** to **break down excess bilirubin.**
4. May require transfusion weeks to months after birth in rare cases

D. HDN Caused by Other IgG Antibodies (Kidd, Kell, etc.)

1. **Any IgG antibody** that can cross the placenta can cause HDN.
2. May be severe and require intrauterine or exchange transfusion
3. **Antibody titration**
   a. Used to predict severity of HDN
   b. Titer needs to be determined as soon as possible in pregnancy.
   c. Repeat titers on positive mothers at 16 and 22 weeks, then every 1–4 weeks until delivery.
   d. A twofold rise in titer indicates a serious situation, and invasive procedures or an exchange transfusion may be necessary.
4. **Amniocentesis** performed
   a. Bilirubin in the amniotic fluid is measured by the change over expected absorbance of the fluid at 450 nm.

E. Laboratory Testing for Predicting Hemolytic Disease of the Newborn

1. ABO and D on mother prior to delivery
2. Antibody screen on mother; infants do not produce antibodies
3. Amniocentesis may be used periodically to monitor hemolytic severity during pregnancy.

F. Suspected Cases of Hemolytic Disease of the Newborn

1. **Cord blood** of infants, born to **D-negative mothers** and in suspected cases of HDN, must be tested for
   a. ABO
   b. D
   c. DAT

G. Prevention of Hemolytic Disease of the Newborn

1. **Prenatal Rh immune globulin (RhIG)** administered to **D-negative women at 28 weeks** (300 μg) and **at childbirth.**
2. One vial of RhIG should be administered to D-negative women after any potential risk of fetal-maternal bleed (i.e., abortions, ectopic pregnancies, amniocenteses, chorionic villus sampling, percutaneous umbilical blood sampling, intrauterine transfusions, and abdominal trauma).
3. **Postpartum administration**
   a. **D-negative women** who give birth to a **D-positive infant** need a 300-μg dose of **RhIG within 72 hours of delivery.**
   b. One **300-μg dose** of RhIG will **neutralize** up to 15 mL RBCs (30 mL whole blood) of **feto-maternal hemorrhage.** If the feto-maternal hemorrhage is >15 mL RBCs, more than one dose is required to neutralize the RBCs.
4. **Fetal screen (Rosette test):** A suspension of maternal RBCs is incubated with anti-D. Anti-D binds to Rh-positive fetal RBCs, if present in the maternal circulation. D-positive indicator cells are added that bind to the anti-D, forming a rosette around the sensitized Rh-positive fetal RBCs. This is a **screening method** to **detect fetomaternal bleeds >15 mL.** If the fetal screen is positive, a Kleihauer-Betke test is required to quantify the amount of bleed that has occurred.
5. **Kleihauer-Betke (KB) acid elution** is used to **determine** the **amount of a fetomaternal hemorrhage.** Principle: Fetal hemoglobin is resistant to acid elution. A blood smear from the mother is made, then dipped in an acid buffer and stained with a counterstain. The buffer lyses the mother's cells (ghost cells) and does nothing to the fetal cells. Pink fetal cells are counted. Results are reported as percent of fetal cells (# fetal cells ÷ total cells counted). The amount, in milliliters (mL), of fetal blood in maternal circulation equals the % fetal cells ×50. Divide the mL of cells by 30 to determine the number of Rh immune globulin doses needed. *Note:* Flow cytometry assays have been developed that can replace the traditional Kleihauer-Betke test.

H. Exchange Transfusions

1. Selection of blood for exchange transfusion
   a. Infant cells must be tested for ABO and D. ABO group of RBCs chosen for transfusion must be compatible with mother's ABO group. Group O blood is typically used.
   b. Mother's blood is used for antibody screen.
   c. Units must be antigen negative for all antibodies in mother's blood.
2. FFP is used to reconstitute packed RBCs to a hematocrit of approximately 40–50%. Group AB FFP is typically used.
3. Any blood products to be transfused must be hemoglobin S negative, CMV negative, and irradiated.

XI. BLOOD COLLECTION

A. Donor Selection

1. Registration questions include full name, address, home and work phone numbers, date of birth, gender, date of last donation, written consent, photo identification, race (optional), and intended use of donation.
2. Educational material is distributed to the donor. The donor must read material, and if the prospective donor shows symptoms of an infectious disease, the donor is excluded from donation.
3. **Donor history questions include:**
   a. Have you ever donated or attempted to donate blood using a different (or another) name here or anywhere else?
   b. In the past 8 weeks, have you given blood, plasma, or platelets here or anywhere else?
   c. Have you for any reason been deferred or refused as a blood donor or told not to donate blood?
   d. Are you feeling well and healthy today?
   e. In the past 12 months, have you been under a doctor's care or had a major illness or surgery?
   f. Have you ever had chest pain, heart disease, recent or severe respiratory disease?
   g. Have you ever had cancer, a blood disease, or a bleeding problem?
   h. Have you ever had yellow jaundice, liver disease, viral hepatitis, or a positive test for hepatitis?
   i. Have you ever had malaria, Chagas disease, or babesiosis?
   j. Have you ever taken etretinate (Tegison) for psoriasis?
   k. In the past 3 years, have you taken acetretin (Soriatane)?
   l. In the past 3 days, have you taken piroxicam (Feldene), aspirin, or anything that has aspirin in it?
   m. In the past month, have you taken isotretinoin (Accutane) or finasteride (Proscar) (Propecia)?

n. In the past 4 weeks, have you taken any pills or medications?
o. In the past 12 months, have you been given rabies shots?
p. Female donors: In the past 6 weeks, have you been pregnant or are you pregnant now?
q. In the past 3 years, have you been outside the United States or Canada?
r. Have you ever received human pituitary-derived growth hormone?
s. Have you received a dura mater (or brain covering) graft?
t. Have you or any of your blood relatives ever had Creutzfeldt-Jakob disease or have you ever been told that your family is at an increased risk for Creutzfeldt-Jakob disease?
u. In the past 12 months, have you had close contact with a person with yellow jaundice or viral hepatitis, or have you been given hepatitis B immune globulin (HBIG)?
v. In the past 12 months, have you taken (snorted) cocaine through your nose?
w. In the past 12 months, have you received blood or had an organ or a tissue transplant or graft?
x. In the past 12 months, have you had a tattoo applied, ear or skin piercing, acupuncture, accidental needlestick, or come in contact with someone else's blood?
y. In the past 12 months, have you had a positive test for syphilis?
z. In the past 12 months, have you had or been treated for syphilis or gonorrhea?
aa. In the past 12 months, have you given money or drugs to anyone to have sex with you?
bb. At any time since 1977, have you taken money or drugs for sex?
cc. In the past 12 months, have you had sex, even once with anyone who has taken money or drugs for sex?
dd. Have you ever used a needle, even once, to take drugs that were not prescribed for you by a doctor?
ee. In the past 12 months, have you had sex, even once, with anyone who has used a needle to take drugs not prescribed by a doctor?
ff. Male donors: Have you had sex with another male, even once, since 1977?
gg. Female donors: In the past 12 months, have you had sex with a male who has had sex with another male, even once, since 1977?
hh. Have you ever taken clotting factor concentrates for a bleeding problem such as hemophilia?
ii. In the past 12 months, have you had sex, even once, with anyone who has taken clotting factor concentrates for a bleeding problem such as hemophilia?
jj. Do you have AIDS or have you had a positive test for the HIV virus?
kk. In the past 12 months, have you had sex, even once, with anyone who has AIDS or has had a positive test for the HIV virus?

ll. Are you giving blood because you want to be tested for HIV or the AIDS virus?
mm. Do you understand that if you have the AIDS virus, you can give it to someone else even though you may feel well and have a negative AIDS test?
nn. Were you born in, or have you lived in, or have you traveled to any African country since 1977?
oo. When you traveled there, did you receive a blood transfusion or any other medical treatment with a product made from blood?
pp. Have you had sexual contact with anyone who was born in or lived in any African country since 1977?
qq. In the past 12 months, have you been in jail or prison?
rr. Have you read and understood all the donor information presented to you, and have all your questions been answered?

4. Examples of **donor deferrals:** See Table 5–5■.
5. All **donors** must pass a **physical exam** with the following criteria:
   a. Appear to be in good health
   b. 38% hematocrit (minimum)
   c. 12.5 g/dL hemoglobin (minimum)
   d. Body temperature must be below 99.5°F (37.5°C).
   e. Blood pressure must be below or equal to 180/100 mm Hg.
   f. Pulse must be between 50 and 100 bpm and regular.
   g. Weight must be a minimum of 110 pounds.
6. **Confidential unit exclusion** (optional)
   a. This is used to give **donors** a way to indicate if this **unit** should be **used for transfusion or discarded.** The most common way to accomplish this is to give the donor **two bar-coded labels:** One states that the blood is OK to use and the other states that the blood should not be used. The donor chooses the label and applies it to his/her records. Once the label is pulled from the backing, the only way of knowing which label is on the records is to scan the bar code.
7. **Informed consent:** The donor must sign a form that allows blood to be collected and used for transfusion.

B. Phlebotomy

1. **Identification** is a crucial step. The donor must be identified before phlebotomy can be done.
2. **Bag labeling:** The bag, attached satellite bags, sample tubes, and donor registration must have the same unique identification number. The labels consist of letters and bar codes.
3. **Postdonation care:** After donating, donors are urged to avoid alcohol and smoking immediately, drink lots of fluid for the next 3 days, and be aware that dizziness and fainting can occur a few hours after donation.

TABLE 5-5 POSSIBLE REASONS FOR DONOR DEFERRALS		
a.	Hepatitis B IgG	12 months
b.	Tattoo/piercing	12 months
c.	Exposure to blood	12 months
d.	Sexual contact with a person at high risk for HIV	12 months
e.	Imprisonment (>72 hours)	12 months
f.	Postblood transfusion	12 months
g.	Rape victim	12 months
h.	Aspirin and aspirin-containing drugs	72 hours
i.	Human pituitary growth hormone injection	indefinite
j.	Sexual contact with anyone who used a needle to take illegal drugs	indefinite
k.	Taken clotting factors	indefinite
l.	AIDS or HIV positive	indefinite
m.	Males having sex with other males	indefinite
n.	Had viral hepatitis	indefinite
o.	Positive HBsAg	indefinite
p.	Positive HBc	indefinite
q.	Positive HTLV-I or HTLV-II	indefinite
r.	History of Creutzfeldt-Jakob disease	indefinite
s.	History of Chagas disease or babesiosis	indefinite

C. Special Blood Collection

1. **Autologous donation:** A donation of blood given by a person to be used for transfusions on themselves at a later date. There are four types—preoperative, intraoperative hemodilution, intraoperative collection, and postoperative collection.
   a. **Advantages:** No diseases transmitted, no alloantibodies formed, no transfusion reactions possible

   b. **Disadvantages:** High waste amount (unused if surgery postponed), adverse donor reactions, and increased cost
2. **Preoperative collection**
   a. Blood is drawn and stored before surgery.
   b. Used for stable patients having a surgery that may require a transfusion
   c. Especially good for patients with existing alloantibodies for whom it may be difficult to find compatible units
   d. Process begins with a physician's order.
   e. Patients must sign informed consent.
   f. Not asked detailed questions about high-risk behavior
   g. Facility makes policy regarding patient's health, age, weight, etc. Hemoglobin should not be below 11 g/dL or hematocrit below 33%.
   h. Blood not drawn sooner than every 72 hours and not drawn within 72 hours of surgery.
   i. Patient's name, transfusion facility, unique patient identification number, expiration date, and "For Autologous Use Only" or "Autologous Donor" tag is on the bag.
   j. ABO and D must be performed at the collecting facility. These tests must be repeated if the transfusing facility is different from the collecting facility.
   k. If transfused outside of the facility, HBsAg, anti-HBc, hepatitis C antibody, HIV 1/2 antigen and antibody, and serologic testing for syphilis must be performed before shipping.
   l. If donor is positive for any of the above, physician's permission is required to use the unit, and a biohazard sticker is attached to the unit before shipping.
   m. An autologous unit cannot be used for allogeneic transfusion; if it is not used by the donor, it must be discarded.
3. **Intraoperative hemodilution (acute normovolemic hemodilution)**
   a. One to two units of the patient's blood are removed at the beginning of surgery and replaced by volume expanders.
   b. Units must be labeled with patient's name, unique identification number, date and time of phlebotomy, and "For Autologous Use Only."
   c. This blood can be stored at room temperature for up to 8 hours or at 1–6°C for 24 hours.
4. **Intraoperative collection (intraoperative salvage)**
   a. Blood lost into the abdominal cavity is collected by a machine. It is washed with saline and transferred back into patient. Blood should not be used if blood will be contaminated with bacteria, as in peritonitis.
5. **Postoperative collection**
   a. Collect blood from surgical drains and deliver into sterile containers.
   b. Collected blood must be transfused within 6 hours.
6. **Directed donations**
   a. Patients choose their own donors.
   b. All AABB *Standards* for donation apply to directed donations.

c. Policies about switching units from directed donation to general donor pool vary among institutions.

7. **Hemapheresis**
   a. **Leukopheresis:** Only WBCs removed from donor blood
   b. **Plateletpheresis:** Only platelets removed from donor blood
   c. **Plasmapheresis:** Only plasma removed from donor blood
   d. **Red cell pheresis:** Only red cells removed from donor
   e. **Apheresis instrument:** An electronic instrument that takes blood from a donor, separates the desired component, and returns the remaining components to the donor. (Process takes from 20 minutes to 2 hours.)
   f. All AABB *Standards* for donation apply to apheresis donors also. However, frequency of donation and additional testing are different for the three types of apheresis:
      1) **Plateletpheresis:** Platelet count of 150,000/μL; 48 hours required between donations, up to 24 times/year
      2) **Leukopheresis:** Not more than twice a week, 24 times/year
      3) **Plasmapheresis:** Every 4 weeks; total protein, IgG, and IgM monitored
      4) **Red cell pheresis:** Every 16 weeks
8. **Therapeutic phlebotomy**
   a. One unit of blood is removed from a patient in a specified time interval.
   b. This is done to treat patient symptoms in polycythemia, hemochromatosis, and porphyria.

XII. BLOOD COMPONENTS: PREPARATION, STORAGE, AND SHIPMENT

A. Definitions

1. **Whole blood:** Blood collected from donors contains all cellular and liquid elements.
2. **Components:** Parts of blood used for treating patients, including RBCs, plasma, platelets, and cryoprecipitated antihemophiliac factor
3. **Hemotherapy:** Use blood or blood components to treat a disease in a patient

B. Blood Collection Bag

1. It is a **closed system** consisting of main bag with needle, tubing, and up to four satellite bags attached. The entire system is **sterile.**
2. **Standard phlebotomy** = 450 mL ± 45 mL or 500 mL ± 50 mL

C. Anticoagulant Preservative Solutions

1. **Standard volume:** 63 mL for 450 mL collections or 70 mL for 500 mL collections
2. If an autologous unit is drawn on a patient weighing less than 110 pounds, the anticoagulant must be reduced.
   a. Reduced Volume Factor (A) = weight of patient ÷ 110 lb
      A × 70 mL = amount of anticoagulant needed (B)

70 − B = amount of anticoagulant to remove
A × 500 mL = amount of blood to collect

b. Example: 90-lb donor
90 lb ÷ 110 lb = 0.81 = A
0.81 × 70 mL = 56.7 mL = B
70 mL − 56.7 mL = 13.3 mL of anticoagulant to be removed from bag
0.81 × 500 mL = 405 mL of blood to be collected

3. **Types of anticoagulants and preservatives**
   a. **Adenine:** Used in ATP synthesis
   b. **Citrate:** Chelates calcium to prevent coagulation
   c. **CPD:** Citrate-phosphate-dextrose
   d. **CP2D:** Citrate-phosphate-2-dextrose
   e. **CPDA-1:** Citrate-phosphate dextrose adenine-1
   f. **Dextrose:** Sugar to support RBC life
   g. **Sodium biphosphate:** Buffer to prevent decreased pH
4. **Storage**
   a. **Shelf life:** This is the amount of storage that blood can take that yields at least 75% of original RBCs still in recipient's circulation 24 hours after transfusion. Remember, blood is still "alive" when it is in a blood bag.
   b. Glucose, ATP, 2,3-BPG, and pH decrease as RBCs are stored. After cells are transfused, ATP and 2,3-BPG levels are restored in about 24 hours.
   c. Substances that increase during storage are all metabolic end products such as potassium, hydrogen ions, etc.
5. **Additive solutions**
   a. AS-1 contains mannitol.
   b. AS-3 contains citrate and phosphate.
   c. AS-5 contains mannitol.
   d. These must be added within 72 hours of collection.
   e. Usually, an additive solution is added to RBCs after plasma is separated off.
   f. Additives extend the shelf life to **42 days** and reduce RBC viscosity during transfusion.
6. **Rejuvenation solution**
   a. Contains phosphate, inosine, pyruvate, and adenine
   b. Its purpose is to restore 2,3-BPG and ATP levels before freezing or transfusing a unit.
   c. May be necessary for autologous or rare units
   d. RBCs can be rejuvenated up to 3 days past the expiration date and can then be frozen for future use.
   e. RBCs can be rejuvenated, stored up to 24 hours at 1–6°C, and transfused. The cells must be washed before transfusion to remove the inosine.
7. **Blood component preparation**
   a. Whole blood is centrifuged and can be separated into RBCs, platelets, fresh-frozen plasma (FFP), and cryoprecipitated antihemophiliac factor.

b. **Process:** Whole blood bag is centrifuged; plasma is separated off into a satellite bag. If platelets are to be prepared from whole blood, two spins are required. The first centrifugation will be a "soft" spin, leaving platelets suspended in the plasma layer. If platelets will not be produced, a single "hard" spin (increased time and rotations per minute [rpm]) will be performed.
c. AS-1 is put into RBC bag (if additive solution is used).
d. RBC bag is sealed and removed from system.
e. Plasma bag is centrifuged to sediment platelets ("hard" spin).
f. Plasma is separated into FFP bag, leaving platelets with 40 to 70 mL of plasma in platelet bag.
g. Platelet bag is sealed off and cut.
h. Plasma is either frozen to make FFP within 8 hours of collection or frozen and later thawed in refrigerated conditions to make cryoprecipitate and cryo-poor plasma.

8. **Storage temperature and expiration dates for components**
   a. **Whole blood:** Storage 1–6°C; expires with CPD, CP2D anticoagulants in 21 days, with CPDA-1 anticoagulant in 35 days, with Adsol (AS-1, AS-3, or AS-5) in 42 days
   b. **RBCs:** Storage 1–6°C; expires with CPD, CP2D anticoagulants in 21 days, with CPDA-1 in 35 days, with AS-1, AS-3, and AS-5 in 42 days
   c. **Platelets:** Storage 20–24°C with rotation, expires in 5 days
   d. **FFP:** Storage –18°C, expires in 1 year; storage –65°C, expires in 7 years
   e. **Cryoprecipitate:** Storage –18°C, expires in 1 year
   f. **RBCs (frozen):** Storage –65°C, expires in 10 years
   g. **RBCs (deglycerolized, washed):** Storage 1–6°C, expires in 24 hours after thawing (deglycerolization)
   h. **RBCs (irradiated):** Storage 1–6°C, expires in 28 days or on originally assigned outdate, whichever comes first
   i. **Platelets (pooled):** Storage 20–24°C, expires in 4 hours after pooling
   j. **Cryoprecipitate (pooled):** Storage 20–24°C, expires 4 hours after pooling
   k. **FFP (thawed):** Storage 1–6°C, expires in 24 hours
   l. **Plateletpheresis:** Storage 20–24°C, expires in 5 days
   m. **Granulocyte pheresis:** Storage 20–24°C, expires in 24 hours

D. Storage and Transportation

1. FDA requirements and AABB *Standards* define calibration and maintenance procedures, storage temperature limits, and monitoring parameters for equipment used to store blood products.
2. **All refrigerators, freezers, and platelet incubators must have**
   a. Recording devices that monitor the temperature at least every 4 hours
   b. Audible alarms that ensure response 24 hours a day
   c. Regular alarm checks
   d. Power failure and alarm activation emergency procedures

e. Emergency power backups (continuous power source for alarms)
f. Calibrated thermometers that are checked against referenced thermometers
g. Written procedures for all the above

3. **Transportation**
   a. Temperature for **RBCs** of **1–10°C** is required during transport. A predetermined amount of wet ice in plastic bags is placed on top of the blood units to maintain the temperature for 24 hours.
   b. RBCs are packed in cardboard boxes with a styrofoam box inside. The ice is double-bagged and weighs approximately nine pounds.
   c. Frozen components are shipped on dry ice. These should be well wrapped because dry ice evaporates, and space in the box for movement should be allowed.
   d. **Platelets** are shipped at **room temperature.** Platelets can survive without agitation for a maximum of 24 hours.
   e. When component shipments are received, observe and record the temperature and appearance of units. If temperature is out of range, units must be evaluated before transfusion. Institutions have policies for determination of the disposition of the units. All problems and dispositions must be documented and stored with blood bank records.

E. Administration of Blood Components

1. Positive identification of patient, sample, and crossmatched unit
2. Only **normal saline** should be infused with blood components.
3. A standard **170-micron filter** must be used with all blood components. **Leukoreduction filters** may be used to reduce the number of leukocytes transfused with RBCs.
4. The maximum transfusion time allowed for one unit to be transfused is **2–4 hours.** If the unit cannot be completely infused within 4 hours, the unit should be divided into two satellite bags and transfused as two separate units.
5. Documentation and accurate recordkeeping are vital.

XIII. BLOOD COMPONENT THERAPY

A. Whole Blood

1. Used in actively bleeding patients, patients who have lost at least 25% of their blood volume, or patients requiring exchange transfusions
2. When whole blood is not available, reconstituted whole blood (RBCs mixed with thawed type AB FFP from a different donor) may be used.

B. RBCs

1. Used in oncology patients undergoing chemotherapy or radiation therapy, trauma patients, surgery patients, dialysis patients, premature infants, and patients with sickle cell anemia

2. **Transfusing one unit** usually **increases** the patient's **hemoglobin** approximately **1 g/dL** and the **hematocrit by 3%.**

C. Leukocyte-Reduced RBCs

1. Used in chronically transfused patients or patients having known febrile transfusion reactions
2. The standard 170-micron filter does not remove leukocytes. A special filter is required for bedside filtration. Leukoreduction (filtration) can also occur in the manufacturing process, which typically occurs within 72 hours from the time of collection.
3. AABB *Standards* for **leukocyte reduction** states that **85% of RBCs must remain** and **leukocytes** must be **reduced to less than 5 $\times$ 10^6 WBC/unit.**

D. Frozen RBCs

1. **Method:** RBCs are frozen by adding **glycerol** to prevent cell hydration and the formation of ice crystals that can cause cell lysis (40% weight per volume).
2. The unit is transferred to a polyolefin or polyvinyl chloride bag, and then the bag is placed in a metal or cardboard canister.
3. **Initial** freezing temp is **–80°C,** then for **long-term storage at –65°C for 10 years.**

E. Deglycerolized RBCs

1. Frozen RBCs are thawed, and then the glycerol must be removed.
2. **Deglycerolization:** Glycerol is drawn out of the RBCs by washing the RBCs with a series of saline solutions of decreasing osmolality.
3. Deglycerolization involves entering the bag, so the **deglycerolized RBCs expire in 24 hours.**

F. Washed RBCs

1. Used for patients who have a reaction to plasma proteins (allergic, febrile, and/or anaphylactic)
2. Used in infant or intrauterine transfusions
3. 10–20% of RBCs are lost in the process of washing the RBC unit with normal saline.

G. Irradiated RBCs

1. **T cells** can cause **graft-versus-host disease,** with 90% of cases being fatal.
2. Gamma irradiation **prevents T cell proliferation.**
3. AABB *Standards* require irradiation of cellular components (RBCs and platelets), if a donor is a blood relative of the intended recipient or donor unit is HLA matched for recipient. Recommended minimum dose of **gamma irradiation** is **25 Gy** (2500 rads).

4. Used for intrauterine transfusions, immunodeficient recipient, premature infants, chemotherapy and radiation patients, and bone marrow or progenitor cells transplant patients

H. Platelets

1. **Purpose:** Used to **control or prevent bleeding**
2. Not indicated in patients with ITP (idiopathic thrombocytopenia)
3. Indicated in patients with chemotherapy, post–bone marrow transplant patients, or patients experiencing postoperative bleeding
4. **Transfused platelets** have a **life span** of **3 to 4 days.**
5. **No crossmatch necessary,** but ABO type-specific preferred.
6. **Platelet concentrates**
   a. Prepared from whole blood unit
   b. **Contain approximately 5.5 $\times$ 10^{10} platelets/unit**
   c. **Raise platelet count by 5000 µL/unit after transfusion**
7. **Pooled platelets**
   a. Procedure is to choose one platelet bag of those to be pooled and empty content of other bags into it.
   b. Usual platelet order is **6–10 units.**
   c. **Opening the unit reduces** the **shelf life** of the bag **to 4 hours.** Platelets should be pooled immediately before transfusion.
8. **Plateletpheresis**
   a. HLA-matched patients who receive numerous platelet transfusions can develop antibodies to the class I HLA antigens on platelets. These patients require HLA matching before transfusion. If platelets to be transfused are not HLA matched, the platelets will not last for 5 days in the patient's circulation.
   b. **Plateletpheresis packs contain approximately 3 $\times$ 10^{11} platelets per unit.**
9. **Leukocyte-reduced platelets**
   a. Filters can reduce the number of leukocytes in a bag while being transfused.
   b. Specific apheresis instruments can reduce leukocyte numbers during collection.

I. Fresh-Frozen Plasma

1. **Purpose:** To **replace coagulation factors** in the patient
2. Indicated in:
   a. Bleeding patients who require factors II, V, VII, IX, and X
   b. Abnormal coagulation due to massive transfusion
   c. Patients on anticoagulants who are bleeding or require surgery
   d. Treatment of TTP and hemolytic uremic syndrome
   e. Patients with liver disease to prevent or correct bleeding

f. Antithrombin III deficiencies
g. DIC when fibrinogen is >100 mg/dL

3. **Thawing**
   a. Thawed in water bath at **30–37°C** for **30–45 minutes** before transfusion
   b. Unit should be placed in watertight container before immersing in water bath to keep ports clean and prevent contamination.
   c. Water baths with agitators are preferred because the unit thaws faster.
   d. FDA-approved microwaves can also be used.

J. Cryoprecipitated Antihemophilic Factor (Cryoprecipitate)

1. Insoluble precipitate is formed when FFP is thawed between 1 and 6°C. It contains factor VIII, fibrinogen, factor XIII, and von Willebrand factor.
2. It is used for patients with **factor XIII deficiency, von Willebrand disease, and fibrinogen deficiency, and as a fibrin sealant.** *Note:* Patients with Factor VIII deficiency are routinely treated with Factor VIII concentrates.
3. Each unit must contain at least **150 mg/dL of fibrinogen** and **80 IU of factor VIII.**
4. **Pooled cryoprecipitate**
   a. Like platelets, cryoprecipitate is pooled into one bag before transfusion.
   b. Units are thawed in a similar fashion to FFP before pooling.
   c. Cryoprecipitate must be given within **4 hours** after pooling.
   d. **Formula** for figuring **factor VIII** in cryoprecipitate:

$$\#\text{ of units} = \frac{\text{plasma volume} \times (\text{desired level \%} - \text{initial level \%})}{80\text{ IU/bag}}$$

   e. Fibrin glue from cryoprecipitate: 1–2 units of cryoprecipitate are mixed with thrombin and applied topically to the bleeding area.

K. Granulocyte Pheresis

1. **Granulocyte transfusions** are rare and limited to **septic infants.**
2. The pheresis bag contains $>1.0 \times 10^{10}$ granulocytes, platelets, and 20–50 mL of RBCs.
3. The cells deteriorate rapidly and must be **transfused within 24 hours** of collection.
4. Store at 20–24°C with no agitation until transfused.
5. **Crossmatching** is **required** because of RBC contamination.

L. Labeling

1. Must conform with Title 21 of the **Code of Federal Regulations** (CFR), specifically 21 CFR 606.120 and 606.121, as well as **FDA** current thinking as described in "Guidance for Industry: Recognition and Use of a Standard for Uniform Blood and Blood Component Container Labels" (9/22/2006). In

addition, facilities accredited by AABB must have implemented **ISBT 128 labeling systems** by May 1, 2008, in accordance with the "United States Industry Consensus Standard for the Uniform Labeling of Blood and Blood Components Using ISBT 128" (November 2005).

2. Current **labeling requirements include** proper name, unique number, amount of blood collected, amount and type of anticoagulant, volume of component, expiration date, storage temperature, ABO/D type, reference to the "Circular of Information for the Use of Human Blood and Blood Components," warning regarding infectious agents, prescription requirements, donor classification, and FDA license number if applicable.
3. Other products must be labeled as follows:
   a. **Irradiated components** must have name of the facility performing the irradiation.
   b. **Pooled components** must include final volume, unique number assigned to the pool, time of expiration, and name of facility preparing the pooled component.
   c. **Autologous units** must be labeled: "For Autologous Use Only."
4. "Circular of Information for the Use of Human Blood and Blood Components": Guidelines that provide a description of each component, indications and contraindications for use, and information of dosage, administration, storage, side effects, and hazards

XIV. TRANSFUSION THERAPY

A. Emergency Transfusions

1. Rapid loss of blood can result in hemorrhagic shock.
   a. **Symptoms:** Hypotension, tachycardia, pallor, cyanosis, cold clammy skin, oliguria, decreased hematocrit, decreased central venous pressure (CVP), CNS depression, and metabolic shock
2. **Priorities in acute blood loss**
   a. Replace and maintain blood volume.
   b. Make sure oxygen-carrying capacity is adequate.
   c. Maintain coagulation system integrity.
   d. Correct metabolic imbalances.
   e. Maintain colloid osmotic pressure.
3. **Massive transfusion:** Replacement of a person's entire blood volume (approximately 10 units) within 24 hours
4. **Emergency transfusions** result from trauma (gunshot wounds, stabbings, vehicular accidents, etc.) and surgical needs.
5. **Emergency release of blood:** It is preferable to transfuse type-specific blood. If time is not available to type the patient, **type O, D-negative** blood is **transfused into women of childbearing age. Type O, D-positive blood** is transfused into **men.** Physician must request emergency release indicating that

no crossmatch is performed before the blood is transfused. The **crossmatch** is **performed during or following the transfusion.**

B. Neonatal and Pediatric Transfusions

1. Smaller blood volume than adults
2. Premature infants may need transfusion to offset the effect of hemoglobin F in their system. Hemoglobin F does not give up oxygen readily.
3. **Iatrogenic blood loss** (blood taken from the neonate or infant for laboratory tests) causes the neonate or infant to develop an anemia that may be severe enough to transfuse.
4. Neonates and infants do not tolerate hypothermia well, so blood warmers may be used.
5. Washed or fresh blood is preferred for neonates or infants because of the liver's inability to metabolize citrate anticoagulants and potassium, which leaks from RBCs in donor units over time.
6. Transfusions are given in small volumes in multiple packs taken from a normal size blood unit.
7. Infants do not form antibodies for the first 4 months, so no crossmatch is necessary.
8. **Transfuse** CMV-negative and/or leukoreduced blood.

C. Transplantation

1. **Liver transplant patients** require large amounts of blood products (on average 20 units of RBCs, 25 units of FFP, 17 units of platelets, and 5 units of cryoprecipitate) because the liver produces many coagulation factors and cholesterol for RBC membranes.
2. **ABO compatibility is important in kidney, liver, and heart transplants.** It is not important in bone, heart valves, skin, and cornea transplants.
3. **Progenitor cell transplants**
   a. **Allogeneic or autologous**
   b. Derived from bone marrow or umbilical cord blood
   c. Transfusion support with leukocyte-reduced products to prevent alloimmunization and a greater chance of rejection
   d. **Conditions treated:** Severe combined immunodeficiency disease, Wiskott-Aldrich syndrome, aplastic anemia, Fanconi anemia, thalassemia, sickle cell disease, acute leukemia, CML, lymphoma, myelodysplastic/myeloproliferative disorders, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, and testicular cancer

D. Therapeutic Hemapheresis

1. Replacement of blood from a patient to improve a patient's health
2. Conditions indicated for therapeutic exchanges: Multiple myeloma, Waldenström macroglobulinemia, hyperleukocytosis, TTP/HUS, sickle cell, myasthenia gravis, acute Guillain-Barré syndrome

E. Oncology

1. Chemotherapy drugs kill all cells that are undergoing mitosis: Stem cells, gastrointestinal epithelial cells, and hair follicles.
2. Action of chemotherapy drugs:
   a. Stopping DNA replication
   b. Interfering with mRNA production

F. Chronic Renal Disease

1. Dialysis patients have an increased uremic (blood urea nitrogen or BUN) content in blood that alters the RBC shape and causes the cells to be removed from circulation by the spleen.
2. Dialysis itself mechanically destroys RBCs.
3. Nonfunctioning kidneys do not produce erythropoietin to stimulate RBC production.
4. The use of transfusions in dialysis patients has been dramatically reduced since erythropoietin therapy was initiated.

G. Sickle Cell Anemia

1. An abnormal hemoglobin (e.g., Hgb S) causes cells to be removed from circulation, resulting in a lowered hematocrit.
2. Because these patients require many transfusions, phenotypically matched units are preferred.
3. Severe cases may be treated by bone marrow transplants.

H. Thalassemia

1. Decreased synthesis of the α- and β-globin chains
2. Hemolytic anemia results
3. Transfusion support necessary

I. Aplastic Anemia

1. Blood transfusion support is usually needed until bone marrow transplant can occur.

XV. TRANSFUSION REACTIONS

A. Types of Transfusion Reactions

1. Transfusion reactions are an adverse physiological reaction to the infusion of blood.
   a. **Hemolytic:** This is a reaction that destroys the transfused blood cells *in vivo*. Large amounts of free hemoglobin are released into the blood and can cause systemic damage.
   b. **Nonhemolytic:** Febrile and allergic
2. **Acute reactions** occur rapidly, within hours of transfusion.
3. **Delayed reactions** occur days or weeks after transfusion.

4. **Immune-mediated transfusion reactions** are due to RBC or HLA antigens and antigen-antibody reactions.
5. Transfusion reactions can also be caused by bacteria, viruses, or parasitic organisms.

B. Hemolytic Transfusion Reactions

1. May be either acute or delayed
   a. **Intravascular** reactions are usually acute, whereas **extravascular** reactions are usually delayed.
   b. Symptoms are variable; they may not be correlated with type of hemolysis.
2. **Mechanism**
   a. **Antibody binding** to RBCs
      1) **Intravascular hemolysis:** IgM antibodies activate the classical pathway of complement that lyses RBCs intravascularly. The lysis releases hemoglobin and RBC remnants into the blood. The excess hemoglobin binds to haptoglobin. Haptoglobin can only bind so much hemoglobin, so the excess hemoglobin is found in the blood and urine.
      2) **Extravascular hemolysis:** Antibody-coated RBCs are removed from circulation by the liver and spleen. The cells lyse when sequestered and, subsequently, bilirubin is released into the blood. Antibodies responsible for this type of hemolysis do not activate the complement cascade or only partially activate it.
   b. **Anaphylatoxins** cause hypotension by triggering serotonin and histamine release.
   c. **Cytokine activation:** Sensitized RBCs are cleared from the blood by phagocytes. The phagocytes release cytokines that cause fever, hypotension, and activation of T- and B cells.
   d. **Coagulation activation:** Antigen-antibody-complement complexes activate the clotting system and cause DIC.
   e. Renal failure is caused by systemic hypotension, reactive renal vasoconstriction, and intravascular thrombi.

C. Acute and Delayed Hemolytic Transfusion Reactions

1. **Acute hemolytic transfusion reactions**
   a. **Clinical signs/symptoms:** Severe, rapid onset, fever, chills, flushing, pain at site of infusion, tachycardia, hemoglobinemia, hemoglobinuria, hypotension
   b. **Major sequelae:** DIC, renal failure, irreversible shock, death
   c. **Mechanisms:** Antigen-antibody reaction activates complement or coats RBCs (i.e., ABO incompatible blood and antibodies to Vel or PP_1P^k antigens)
   d. **Occurrence:** 1:25,000 transfusions
   e. **Most common cause:** Identification error in patient, unit, and/or specimen
   f. **Diagnostic laboratory tests:** Elevated plasma free hemoglobin, elevated bilirubin (6 hours posttransfusion), decreased haptoglobin, and positive DAT

2. **Delayed hemolytic transfusion reactions**
   a. Usually less severe than acute hemolytic transfusion reaction, and dependent on the concentration of antibody in the blood rather than the type of antibody
   b. **Clinical signs:** 5–7 days posttransfusion, fever, mild jaundice
   c. **Major sequelae:** Usually none. However, antibodies in the Kidd system can cause major delayed hemolysis.
   d. **Causes:** Alloantibodies to Rh, Duffy, and Kidd antigens; patient with low concentration of alloantibody experiences anamnestic response when reexposed to RBC antigen
   e. **Occurrence:** 1:2,500 transfusions
   f. **Diagnostic laboratory tests:** Positive DAT, positive posttransfusion antibody screen, and decreased hemoglobin and hematocrit

D. Causes of Non-Immune-Mediated Mechanisms of RBC Destruction

1. Transfusion of hemolyzed units
2. Malfunctioning or unregulated blood warming units
3. Improper thawing and deglycerolization of a frozen RBC unit
4. Physical destruction by needles, valves, or equipment
5. RBC defects
6. Administration of drugs and/or non-isotonic solutions with blood unit

E. Immune-Mediated Nonhemolytic Transfusion Reaction

1. **Clinical signs**
   a. Fever with temperature increase 1°C over baseline temperature 8–24 hours posttransfusion
   b. Nausea, vomiting, headache, and back pain
2. **Causes:** HLA antibody in recipient to donor antigens; cytokines in blood products containing WBCs and platelets
3. **Occurrence**
   a. Common in patients with multiple pregnancies and transfusions
   b. Multiple exposures to HLA antigens
   c. Common in women
   d. 1:200 donor units transfused

F. Allergic Transfusion Reactions

1. **Urticarial reactions**
   a. Clinical signs: Wheals, hives, itching
   b. Sequelae: None
   c. Causes: Recipient forms antibodies to foreign proteins in donor plasma
   d. Occurs in 1–3% of recipients
2. **Anaphylactic reactions**
   a. Clinical signs: Rapid onset, severe wheezing and cough, and bronchospasms

b. Sequelae: Syncope, shock, death
c. Cause: Genetic IgA deficiency
d. Occurs very rarely

G. Transfusion-Associated Graft-versus-Host Disease

1. Clinical signs: 3–30 days posttransfusion, fever, erythematous maculopapular rash, abnormal liver function
2. Sequelae: Sepsis, hemorrhage, 90% mortality rate
3. Cause: Transfused T cells react against recipients
4. Occurs rarely

H. Bacterial Contamination of Blood Products

1. Bacterial contamination usually occurs during phlebotomy or during thawing of frozen blood components.
2. Bacteria (*Yersinia enterocolitica,* most common) live and multiply in bag during storage.
3. Bacterial endotoxins can be present in the unit of blood and cause symptoms similar to hemolytic transfusion reactions.
4. 2% of units are contaminated.
5. Workup: Blood cultures drawn from patient; gram stain and culture of the unit
6. Person issuing unit needs to check for discoloration, clots, cloudiness, or hemolysis before unit is released.

I. Circulatory Overload

1. Too much blood in a patient's vascular system caused by transfusing a unit too fast; most often occurs in children and elderly patients
2. Symptoms: Dyspnea, severe headache, peripheral edema, and signs of congestive heart failure occurring after transfusion; can be fatal

J. Other Complications

1. **Hemosiderosis:** This condition, which is characterized by the deposition of the iron-containing pigment hemosiderin in organs such as the liver and spleen, occurs in chronically transfused patients, especially those with hemolytic anemias.
2. **Citrate overload:** Massive transfusions introduce large amounts of citrate into the body. Citrate binds ionized calcium, but it can be alleviated by calcium chloride or calcium gluconate injections.

K. Transfusion Protocol and Suspected Transfusion Reaction Workup

1. Transfusionist checks and rechecks all paperwork, requisition, and blood bag tag before beginning the transfusion to ensure there were no clerical errors made.
2. Vital signs (blood pressure, temperature, respiration, and pulse) are taken before beginning and every 15 minutes for the first hour and then hourly until the transfusion is completed.

3. If a reaction is suspected:
   a. Stop the transfusion.
   b. Notify the physician and the laboratory.
   c. Physician evaluates the patient.
   d. Draw EDTA and red top tubes, and collect first voided urine for laboratory testing according to institutional policy.
4. **Laboratory responsibilities**
   a. Check all samples, requisition, histories, and bags for identical patient identification. **Clerical errors** are responsible for most transfusion reactions.
   b. Examine pretransfusion and posttransfusion patient samples for hemolysis.
   c. Perform **DAT** on posttransfusion patient sample. If the posttransfusion sample is positive, the DAT is then performed on the pretransfusion sample.
   d. If clerical errors are eliminated and pre- and post-transfusion patient samples show no hemolysis and have negative DAT, the workup is considered to be not indicative of a hemolytic transfusion reaction.
   e. If any positive DAT or hemolysis is found in posttransfusion samples that was not present in pretransfusion samples, further testing is required. Repeat ABO and D on pretransfusion patient sample, posttransfusion patient sample, and segments from the bag; repeat antibody screen and crossmatch on old and new patient samples. Other tests may include hemoglobin, hematocrit, haptoglobin, urine hemoglobin, and bilirubin.
5. **Transfusion reaction workup records**
   a. Must be retained in the blood bank indefinitely
   b. Bacterial contamination and transmitted diseases are reported to blood collection facility.
   c. Fatalities are reported to FDA's Office of Compliance, Center for Biologics Evaluation and Research, within 24 hours.

XVI. TRANSFUSION-TRANSMITTED DISEASES

A. Donor Infectious Disease Testing (Test and Date Testing Started)

1. HBsAg (before 1980)
2. HBc antibody (1986)
3. HCV antibody (1990); HCV NAT testing (1999 under IND/licensed in 2002)
4. HIV-1/2 antibody (HIV-1: 1985; HIV-2: 1992)
5. HIV-1 p24 antigen (1996, discontinued 2002); HIV-1 NAT testing (1999 under IND/licensed in 2002)
6. HTLV-I/II antibody (1997)
7. Syphilis (before 1980)

8. CMV (only performed on small portion of inventory; CMV negative blood needed for premature infants, intrauterine transfusion, and immunocompromised recipients)
9. *T. cruzi* antibody/Chagas disease (2007/currently not mandated)
10. West Nile Virus NAT testing (2003 under IND/license 2007)

B. Look-Back Studies

1. FDA requires **notification** of patients who received units from donors that subsequently tested positive for HIV-1/2 or HCV.
   a. Identify any blood products previously donated by a donor currently testing positive.
   b. Identify all blood products donated by that donor 12 months before the last negative screening test.
   c. Notify facilities that received units involved in the look-back investigation.
   d. Trace to patients and notify patients of potential exposure.

XVII. SAFETY AND QUALITY ASSURANCE

A. FDA Regulations

1. Mandate adherence to **Current Good Manufacturing Practice** (cGMP)
   a. Write standard operating procedures.
   b. Follow standard operating procedures.
   c. Record and document all work.
   d. Qualify personnel by training and education.
   e. Design and build proper facilities and equipment.
   f. Clean by following a housekeeping schedule.
   g. Validate equipment, personnel, processes, etc.
   h. Perform preventive maintenance on facilities and equipment.
   i. Control for quality.
   j. Audit for compliance with all the above.

B. Records

1. **Good recordkeeping**
   a. Use permanent ink on documents.
   b. Record data on proper form.
   c. **No white-out correction fluid** is permitted; cross out mistake and have person making correction date and initial it.
   d. No ditto marks used.
   e. Record "broken, closed, or not in use" when appropriate.
2. **Retention (indefinite)**
   a. Donor's identification information, medical history, physical exam, consent, and interpretations for disease markers
   b. Information on blood and components from an outside source, including numeric or alphanumeric identification on old unit and identification of the

collecting facility, needs to be retained. However, the information from an intermediate facility may be used, if the intermediate facility retains the unit number and identification number of the collecting facility.

c. Identification of facilities that carry out any part of the preparation of blood components, and the functions they perform
d. Final disposition of each unit of blood or blood component
e. Notification to donors of permanent deferral
f. Records of prospective donors who have been placed on surveillance or indefinitely deferred for the protection of the potential recipient
g. Notification to transfusing facilities of previous receipt of units from donors subsequently found to be confirmed positive for HIV and human T cell lymphotropic virus type 1 (HTLV)
h. Difficulty in blood typing, clinically significant antibodies, and adverse reactions to transfusions
i. Notification to recipients of potential exposure to disease transmissible by blood
j. Names, signatures, initials, or identification codes and inclusive dates of employment of those authorized to sign or review reports and records

3. **Retention (minimum of 10 years)**
   a. Donor's ABO, D, difficulty in blood typing, severe adverse reactions to donation, and apheresis procedure clinical record
   b. Records of blood component inspection before issue
   c. Patient's ABO and D type, interpretation of compatibility testing, and therapeutic procedures, including phlebotomy, apheresis, and transfusion
   d. All superseded procedures, manuals, and publications
   e. Control testing of components, reagents, and equipment
   f. Proficiency testing surveys, including dates, performed tests, observed results, interpretations, identification of personnel carrying out the tests, and any appropriate corrective actions taken
   g. Documentation of staff qualifications, training, and competency testing
   h. Quality systems audits and internal assessment records

C. Document Control

1. Must be complete, organized, appropriately stored, retrievable, and secure

D. Personnel Qualifications

1. Job descriptions written with specific job duties are required.
2. Selection criteria for an employee must be developed.
3. Training must be provided during new employee orientation and whenever procedures change or the employee performs poorly.
4. Competency assessment means evaluating the skill on a level of knowledge of an employee. This is accomplished through performance observation; written tests; review of results, records, or worksheets; and/or testing unknown samples (i.e., proficiency).

E. Supplier Qualifications

1. Evaluate products and services received from a supplier to see if established criteria are met.

F. Validation

1. Validation ensures that products or services will meet established criteria for a high degree of quality assurance.
2. All blood bank information systems must be validated before being put into use.

G. Federal, State, and Local Safety Regulations

1. FDA
   a. **Biologics Control Act of 1902**
      1) Licensing of manufacturers and products
      2) Labeling
      3) Facility inspections
      4) Suspension or revoking license
      5) Penalties for violation
   b. The Act was expanded in 1944 and implemented under the Public Health Service Act.
2. Occupational Safety and Health Administration (OSHA)
   a. **Occupational Safety and Health Act**
      1) Ensures a safe and healthy workplace
      2) Act enforced by OSHA
   b. Employers must inform employees about OSHA regulations and post OSHA literature that informs employees about their right to know.
   c. Updates to OSHA are published annually in the Code of Federal Regulations (CFR).
3. **Centers for Disease Control and Prevention (CDC)**
   a. CDC introduced universal precautions in 1987 to decrease risks of bloodborne pathogen exposure. Currently, these safety practices are referred to as **"standard precautions."**
   b. In 1991, **OSHA** published the final standard on bloodborne pathogens. This regulation requires:
      1) Hazard-free workplace
      2) Provision of education and training to staff
      3) Evaluation of potential risks
      4) Evaluation of positions for potential risks
      5) Posting of signs and use of labels
      6) Implementation of standard precautions for handling biohazardous substances
      7) Provision of personal protective equipment (PPE), such as gloves, fluid resistant lab coats, and splash shields, at no cost to the employee

8) Provision of free hepatitis B vaccine to at-risk staff
9) Provision of free hepatitis B immunoglobulin for any exposures to employee

XVIII. BLOOD USAGE REVIEW

A. Peer Review: Mandated by The Joint Commission Standards (for accreditation), CFR (for Medicare reimbursement), most states (for Medicaid reimbursement), CAP (for accreditation), and AABB (for accreditation)

1. The Joint Commission requires the medical staff to review blood usage quarterly for:
   a. Appropriateness of transfusions for blood and blood products
   b. Evaluation of transfusion reactions
   c. Development and implementation of policies and procedures for blood product distribution, handling, use, and administration
   d. Adequacy of transfusion services to meet the needs of patients
   e. Blood product ordering practices
2. **Hospital transfusion practice** is usually monitored by the Hospital Transfusion Committee. This committee reviews:
   a. Statistical data (retrospectively, i.e., data collected over a specified period of time)
   b. Physician ordering patterns (retrospectively, i.e., data collected over a specified period of time)
   c. Concurrent review

review questions

INSTRUCTIONS Each of the questions or incomplete statements that follows is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

Blood Collection, Preservation, Processing, Component Preparation, and Quality Control

1. A woman wants to donate blood. Her physical examination reveals the following: weight—110 lb, pulse—73 bpm, blood pressure—125/75 mm Hg, hematocrit—35%. Which of the following exclusions applies to the prospective donor?
   A. Pulse too high
   B. Weight too low
   C. Hematocrit too low
   D. Blood pressure too low

2. A potential donor has no exclusions, but she weighs only 95 pounds. What is the allowable amount of blood (including samples) that can be drawn?
   A. 367 mL
   B. 378 mL
   C. 454 mL
   D. 473 mL

3. Donors who have received blood or blood products within 12 months of when they desire to donate are deferred to protect the recipient because the
   A. Blood could have transmitted hepatitis (HBV or HCV) or HIV
   B. Blood may have two cell populations
   C. Donor may not be able to tolerate the blood loss
   D. Donor red cell hemoglobin level may be too low

4. Which of the following conditions would contraindicate autologous presurgical donation?
   A. Weight of 100 pounds
   B. Age of 14 years
   C. Hemoglobin of 12 g/dL
   D. Mild bacteremia

5. Which of the following donors would be deferred indefinitely?
   A. History of syphilis
   B. History of gonorrhea
   C. Accutane® treatment
   D. Recipient of human growth hormone

6. Which of the following viruses resides exclusively in leukocytes?
   A. CMV
   B. HIV
   C. HBV
   D. HCV

7. A donor indicates that he has taken two aspirin tablets per day for the last 36 hours. The unit of blood
   A. May not be used for pooled platelet concentrate preparation
   B. Should not be drawn until 36 hours after cessation of aspirin ingestion
   C. May be used for pooled platelet concentrate preparation
   D. May be used for red blood cells and fresh-frozen plasma production, but the platelets should be discarded

8. Which of the following best describes what must be done with a unit of blood drawn from a donor who is found to be at high risk of contracting acquired immune deficiency syndrome (AIDS)?
   A. Hold unit in quarantine until donor diagnosis is clarified.
   B. Use the blood for research dealing with AIDS.
   C. Properly dispose of unit by autoclaving or incineration.
   D. Use the plasma and destroy the red blood cells.

9. Which of the following is *least* likely to transmit hepatitis?
   A. Cryoprecipitate
   B. RBC
   C. Plasma protein fraction (PPF)
   D. Platelets

10. A pooled sera product from 16 donors has a repeatedly positive nucleic acid test (NAT) for HCV. The next action that should be taken is to
    A. Permanently exclude all the donors in the pool
    B. Test each donor in the pool for HCV
    C. Label all the donors as HCV positive
    D. Confirm the positive using recombinant immunoblot assay (RIBA)

11. Although cryoprecipitate has primarily been used for treatment of hypofibrinogenemia and hemophilia A, it contains other blood proteins useful in the treatment of coagulopathies. Which of the following is *not* found in cryoprecipitate?
    A. Fibronectin
    B. Factor XIII
    C. Factor VIII:vW
    D. Antithrombin III

12. Even though it is properly collected and stored, which of the following will fresh-frozen plasma (FFP) *not* provide?
    A. Factor V
    B. Factor VIII
    C. Factor IX
    D. Platelets

13. Blood needs to be prepared for intrauterine transfusion of a fetus with severe HDN. The red blood cell unit selected is compatible with the mother's serum and has been leuko-depleted. An additional step that must be taken before transfusion is to
    A. Add pooled platelets and fresh-frozen plasma
    B. Check that the RBC group is consistent with the father's
    C. Irradiate the RBCs before infusion
    D. Test the RBC unit with the neonate's eluate

14. The addition of adenine in an anticoagulant-preservative formulation aids in
    A. Maintaining ATP levels for red cell viability
    B. Maintaining platelet function in stored blood
    C. Reducing the plasma K^+ levels during storage
    D. Maintaining 2,3-BPG levels for oxygen release to the tissues

15. The pilot tubes for donor unit #3276 break in the centrifuge. You should
    A. Label the blood using the donor's previous records
    B. Discard the unit because processing procedures cannot be performed
    C. Discard the red cells and salvage the plasma for fractionation
    D. Remove sufficient segments to complete donor processing procedures

Use the following information to answer questions 16 and 17.

A satellite bag containing 250 mL of fresh plasma is selected for quality control of cryoprecipitate production. Cryoprecipitate is prepared according to standard operating procedures. The final product has a total volume of 10 mL. The factor VIII assays are 1 IU/mL before and 9 IU/mL after preparation.

16. What is the percent yield of factor VIII in the final cryoprecipitate?
    A. 11%
    B. 25%
    C. 36%
    D. 80%

17. Does this product meet AABB *Standards* for cryoprecipitate production?
    A. Yes
    B. No; the percent recovery is too low.
    C. No; the final factor VIII level is too low.
    D. Data are insufficient to calculate.

Use the following information to answer questions 18–21.

A centrifuge used for platelet preparation has been returned after major repair. A unit of whole blood (450 mL; platelet count 200,000/μL) is selected for calibration of platelet production. The platelet-rich plasma (PRP) contains 250 mL with a platelet count of 300,000/μL. The final platelet concentrate prepared from the PRP contains 50 mL with a platelet count of 900,000/μL.

18. What is the percent yield of platelets in the PRP from this unit?
    A. 33%
    B. 45%
    C. 66%
    D. 83%

19. What is the percent yield of platelets in the final product from the PRP?
    A. 30%
    B. 45%
    C. 50%
    D. 60%

20. Does this product meet AABB *Standards* for platelet concentrate production?
    A. Yes
    B. No; the count on the final product is too low.
    C. No; the percentage recovery in the PRP is too low.
    D. Data are insufficient to calculate.

21. The final product was prepared with a PRP spin time of 2 minutes at 2500 rpm. To increase the percent platelet yield in the final product, one would
    A. Increase the time and/or rpm for the first spin
    B. Increase the time and/or rpm for the second spin
    C. Decrease the time and/or rpm for the first spin
    D. Decrease the time and/or rpm for the second spin

22. When 2,3-BPG levels drop in stored blood, which of the following occurs as a result?
    A. Red blood cell K^+ increases.
    B. Red blood cell ability to release O_2 decreases.
    C. Plasma hemoglobin is stabilized.
    D. ATP synthesis increases.

23. The last unit of autologous blood for an elective surgery patient should be collected no later than _____ hours before surgery.
    A. 24
    B. 36
    C. 48
    D. 72

24. For which of the following patients would autologous donation *not* be advisable?
    A. Patients with an antibody against a high-incidence antigen
    B. Patients with uncompensated anemia
    C. Open heart surgery patients
    D. Patients with multiple antibodies

25. It is generally asymptomatic but has a very high carrier rate (70–80% have chronic infections). About 10% of the carriers develop cirrhosis or hepatocellular carcinoma. These statements are most typical of which of the following transfusion-transmitted infections?
    A. HAV
    B. HBV
    C. HCV
    D. HEV

26. Biochemical changes occur during the shelf life of stored blood. Which of the following is a result of this "storage lesion"?
    A. Increase in pH
    B. Increase in plasma K^+
    C. Increase in plasma Na^+
    D. Decrease in plasma hemoglobin

27. It has been determined that a patient has posttransfusion hepatitis and received blood from eight donors. There is nothing to indicate that these donors may have been likely to transmit hepatitis. What action must be taken initially?
    A. Defer all donors indefinitely from further donations.
    B. Repeat all hepatitis testing on a fresh sample from each donor.
    C. Notify the donor center that collected the blood.
    D. Interview all implicated donors.

28. The temperature range for maintaining red blood cells and whole blood during shipping is
    A. 0–4°C
    B. 1–6°C
    C. 1–10°C
    D. 5–15°C

29. Platelets play an important role in maintaining hemostasis. One unit of donor platelets derived from whole blood should yield _____ platelets.
    A. 5.5×10^6
    B. 5×10^8
    C. 5.5×10^{10}
    D. 5×10^{10}

30. The pH of four platelet concentrates is measured on the day of expiration. The pH and plasma volumes of the four units are as follows: pH 6.0, 45 mL; pH 5.5, 38 mL; pH 5.8, 40 mL; pH 5.7, 41 mL. What corrective action is needed in product preparation to meet AABB *Standards* for platelet production?
    A. No corrective action is necessary.
    B. Recalibrate pH meter.
    C. Increase final plasma volume of platelet concentrates.
    D. Decrease final plasma volume of platelet concentrates.

31. During preparation of platelet concentrate, the hermetic seal of the primary bag is broken. The red blood cells
    A. Must be discarded
    B. May be labeled with a 21-day expiration date if collected in CPD
    C. Must be labeled with a 24-hour expiration date
    D. May be glycerolized within 6 days and stored frozen

32. The blood bank procedures manual must be
    A. Revised annually
    B. Revised after publication of each new edition of AABB *Standards*
    C. Reviewed prior to a scheduled inspection
    D. Reviewed annually by an authorized individual

33. Previous records of patients' ABO and Rh types must be immediately available for comparison with current test results
    A. For 6 months
    B. For 12 months
    C. For 10 years
    D. Indefinitely

34. Which of the following weak D donor units should be labeled Rh-positive?
    A. Weak D due to transmissible genes
    B. Weak D as position effect
    C. Weak partial D
    D. All the above

35. In order to meet the current AABB *Standards* for leukocyte reduction to prevent HLA alloimmunization or CMV transmission, the donor unit must retain at least _____ of the original red cells and leukocytes must be reduced to less than _____.
    A. 85%, 5×10^8
    B. 80%, 5×10^6
    C. 75%, 5×10^5
    D. 70%, 5×10^4

36. Which of the following tests is/are *not* performed during donor processing?
    A. ABO and Rh grouping
    B. HB_sAg
    C. HIV-1-Ag
    D. HB_sAb

37. A 70-kg man has a platelet count of 15,000/μL, and there are no complicating factors such as fever or HLA sensitization. If he is given a platelet pool of 6 units, what would you expect his posttransfusion count to be?
    A. 21,000–27,000/μL
    B. 25,000–35,000/μL
    C. 45,000–75,000/μL
    D. 75,000–125,000/μL

38. Which of the following tests on donor red blood cells must be repeated by the transfusing facility when the blood was collected and processed by a different facility?
    A. Confirmation of ABO group and Rh type of blood labeled D-negative
    B. Confirmation of ABO group and Rh type
    C. Weak D on D-negatives
    D. Antibody screening

INSTRUCTIONS: Each numbered group of incomplete statements (questions 39–63) is followed by four suggested responses. Select the *best* answer or completion statement in each case. Lettered responses may be used once, more than once, or not at all.

For the following components prepared from whole blood (questions 39–43), indicate the required storage temperature.

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39. Red blood cells (RBCs), liquid

40. Red blood cells, frozen

41. Fresh-frozen plasma

42. Cryoprecipitate

43. Platelet concentrate

    A. 1–6°C
    B. 20–24°C
    C. –18°C or colder
    D. –65°C or colder

For the following components prepared from whole blood (questions 44–48), indicate the shelf life.

44. Red blood cells in CPDA-1
45. Fresh-frozen plasma
46. Cryoprecipitate
47. Fresh-frozen plasma, thawed
48. Platelet concentrate in PL-732 (with agitation)

A. 24 hours
B. 5 days
C. 35 days
D. 1 year

Using the specified anticoagulant/preservative (questions 49–52), indicate the allowable shelf life for blood for transfusion therapy.

49. CPD (citrate phosphate dextrose)
50. CPDA-1 (citrate phosphate dextrose adenine)
51. AS-1 (Adsol®)
52. EDTA

A. 21 days
B. 35 days
C. 42 days
D. Not an approved anticoagulant

For the following situations (questions 53–59), indicate whether the individual volunteering to donate blood for allogeneic transfusion should be accepted or deferred. Assume results of the physical examination to be acceptable unless noted.

53. A 65-year-old man whose birthday is tomorrow
54. A 45-year-old woman who donated a unit during a holiday appeal 54 days ago
55. A 50-year-old man who had sex with another man in 1980
56. A 25-year-old man who says he had yellow jaundice right after he was born
57. An 18-year-old with poison ivy on his hands and face
58. A woman who had a baby 2 months ago
59. A 35-year-old runner (pulse 46 bpm)

A. Defer temporarily
B. Defer for 12 months
C. Defer indefinitely
D. Accept

For the following patients (questions 60–63), indicate the component of choice for transfusion therapy.

60. Patients with warm autoimmune hemolytic anemia (AIHA) due to α-methyldopa (Aldomet®) with hemoglobins of 8.5 g/dL or above
61. Patients requiring transfusion with RBC that will not transmit cytomegalovirus (CMV)
62. Patients with normovolemic anemia
63. Patients who are thrombocytopenic secondary to the treatment of acute leukemia

A. Platelet concentrate
B. RBC
C. Leukocyte-reduced RBC
D. Transfusion *not* indicated

Blood Groups, Genetics, Serology

64. Most blood group antibodies are of what immunoglobulin classes?
    A. IgA and IgD
    B. IgA and IgM
    C. IgE and IgD
    D. IgG and IgM

65. The following family study is performed:

Mother	Father	Child 1	Child 2
K + k +	K − k +	K + k −	K − k +

All other indications are that these children are both the products of this mating. Possible explanations for these results would include
    A. A dominant inhibitor gene has been passed to child 1
    B. Father has one *k* gene and one K^0 gene
    C. Father has the McLeod phenotype
    D. Mother has a *cis-Kk* gene

66. Which of the following blood groups reacts *least* strongly with an anti-H produced in an A_1B individual?
    A. Group O
    B. Group A_2B
    C. Group A_2
    D. Group A_1

67. How many genes encode the following Rh antigens: D, C, E, c, e?
    A. One
    B. Two
    C. Three
    D. Four

Use the following information to answer questions 68 and 69.

A patient arrives in the OB clinic 3 months pregnant. This is her first pregnancy, and she has never been transfused. Her prenatal screen includes the following results:

Cell Typing Results		Serum Typing Results	
Anti-A	Anti-B	A_1 cells	B cells
0	0	4+	4+

	Screening Cell I	Screening Cell II	Auto-control
IS	4+	4+	0
37°C LISS	4+	4+	0
AHG	4+	4+	0
Check Cells			√

68. The test results could be due to
    A. Cold autoantibody
    B. Inheritance of *sese* genes
    C. Inheritance of *hh* genes
    D. Rouleaux

69. If the patient's RBCs were tested against anti-H lectin and did not react, this person would be identified as a(an)
    A. Acquired B
    B. O_h phenotype
    C. Secretor
    D. Subgroup of A

70. If a person has the genetic makeup *Hh, AO, LeLe, sese,* what substance will be found in the secretions?
    A. A substance
    B. H substance
    C. Le^a substance
    D. Le^b substance

71. The following results were obtained when typing a patient's blood sample.

Cell Typing Results		Serum Typing Results	
Anti-A	**Anti-B**	**A_1 cells**	**B cells**
4+	2+	0	4+

    The tech suspects that this is a case of an acquired B antigen. Which of the following would support this suspicion?
    A. A positive autocontrol test
    B. Secretor studies show that the patient is a nonsecretor.
    C. A patient diagnosis of leukemia
    D. The patient's red cells give a negative result, with a monoclonal anti-B reagent lacking the ES-4 clone.

72. Lectins are useful in determining the cause of abnormal reactions in blood bank serology. These lectins are frequently labeled as anti-H, anti-A_1, etc. The nature of these lectins is explained by which of the following?
    A. An early form of monoclonal antibody produced in nonvertebrates
    B. A plant substance that chemically reacts with certain RBC antigens
    C. Naturally occurring antibodies in certain plants
    D. The ability of plants to respond to RBC antigens by antibody production

73. Which of the following sugars must be present on a precursor substance for A and B antigenic activity to be expressed?
    A. D-Galactose
    B. *N*-Acetylgalactosamine
    C. Glucose
    D. L-Fucose

74. An antigen-antibody reaction alone does not cause hemolysis. Which of the following is required for red blood cell lysis?
    A. Albumin
    B. Complement
    C. Glucose-6-phosphate dehydrogenase (G6PD)
    D. Antihuman globulin (AHG)

75. A white female's red blood cells gave the following reactions upon phenotyping: D+ C+ E– c+ e+. Which of the following is the most probable Rh genotype?
    A. *DCe/Dce*
    B. *DCe/dce*
    C. *DCe/DcE*
    D. *Dce/dCe*

76. A black patient has the following Rh phenotype: D+ C+ E+ c+ e+. Which of the following genotypes is the *least* probable?
    A. *DCE/dce*
    B. *DCe/DcE*
    C. *DCe/dcE*
    D. *DcE/dCe*

77. An individual of the *dce/dce* genotype given *dCe/dce* blood has an antibody response that appears to be anti-C plus anti-D. What is the most likely explanation for this?
    A. The antibody is anti-G.
    B. The antibody is anti-partial D.
    C. The antibody is anti-Cw.
    D. The reactions were read incorrectly.

78. If a patient has the Rh genotype *DCe/DCe* and receives a unit of red blood cells from a *DCe/dce* individual, what Rh antibody might the patient develop?
    A. Anti-C
    B. Anti-c
    C. Anti-d
    D. Anti-E

Use the following information to answer questions 79–81.

The following results were obtained when testing the individuals below:

	Anti-					Test for
	D	C	E	c	e	Weak D
Husband	0	+	0	+	+	+
Wife	0	0	0	+	+	0
Infant	+	0	0	+	+	N/A

79. What percentage of this couple's offspring can be expected to be D-negative?
    A. 0%
    B. 25%
    C. 50%
    D. 75%

80. Which of the following conclusions regarding the family typing is most likely?
    A. The husband is not the infant's father.
    B. The husband is proved to be the infant's father.
    C. The husband cannot be excluded from being the infant's father.
    D. The D typing on the infant is a false positive.

81. Which, if any, of these three individuals can make anti-D?
    A. Husband
    B. Husband and wife
    C. Wife
    D. None

82. If a D-positive person makes an anti-D, this person is probably
    A. Partial D
    B. D-negative
    C. Weak D as position effect
    D. Weak D due to transmissible genes

83. A serum containing anti-k is not frequently encountered. This is because
    A. People who lack the k antigen are rare
    B. People who possess the k antigen are rare
    C. The k antigen is not a good immunogen
    D. $Kell_{null}$ people are rare

84. A victim of an auto accident arrives in the emergency department (ED) as a transfer from a hospital in a rural area. The patient has been in that facility for several weeks and has received several units of red blood cells during that time. The ED resident orders 2 units of RBCs for transfusion. The sample sent to the blood bank is centrifuged and the cell-serum interface is not discernable. A subsequent sample produces the same appearance. You would suspect that the patient has
    A. Autoimmune hemolytic anemia
    B. Anti-Fy^a
    C. Anti-Jk^a
    D. Paroxysmal nocturnal hemoglobinuria

85. Which of the following is a characteristic of the Xg^a blood group system?
    A. The Xg^a antigen has a higher frequency in women than in men.
    B. The Xg^a antigen has a higher frequency in men than in women.
    C. The Xg^a antigen is enhanced by enzymes.
    D. Anti-Xg^a is usually a saline-reacting antibody.

86. Testing needs to be done with an antiserum that is rarely used. The appropriate steps to take in using this antiserum include following the manufacturer's procedure and
    A. Performing a cell panel to be sure that the antiserum is performing correctly
    B. Performing the testing on screen cells
    C. Testing in duplicate to ensure the repeatability of the results
    D. Testing a cell that is negative for the antigen and one that is heterozygous for the antigen

87. Which of the following is a characteristic of Kidd system antibodies?
    A. Usually IgM antibodies
    B. Corresponding antigens are destroyed by enzymes.
    C. Usually strong and stable during storage
    D. Often implicated in delayed hemolytic transfusion reactions

88. Which of the following statements is *not* true of anti-Fy^a and anti-Fy^b?
    A. Are clinically significant
    B. React well with enzyme-treated panel cells
    C. Cause hemolytic transfusion reactions
    D. Cause a generally mild hemolytic disease of the newborn

89. Which of the following antibodies can be neutralized with pooled human plasma?
    A. Anti-Hy and anti-Ge:1
    B. Anti-Ch^a and anti-Rg^a
    C. Anti-Co^a and anti-Co^b
    D. Anti-Do^a and anti-Js^b

90. Which of the following statements is *not* true about anti-U?
    A. Is clinically significant
    B. Is only found in black individuals
    C. Only occurs in S–s– individuals
    D. Only occurs in Fy(a–b–) individuals

91. A patient had an anti-E identified in his serum 5 years ago. His antibody screening test is now negative. To obtain suitable blood for transfusion, what is the best procedure to use?
    A. Type the patient for the E antigen as an added part to the crossmatch procedure.
    B. Type the donor units for the E antigen and crossmatch the E-negative units.
    C. Crossmatch donors with the patient's serum and release the compatible units for transfusion.
    D. Perform the crossmatch with enzyme-treated donor cells, because enzyme-treated red cells react better with Rh antibodies.

92. A patient's red blood cells are being typed for the Fy^a antigen. Which of the following is the proper cell type of choice for a positive control of the anti-Fy^a reagent?
    A. Fy(a+b–)
    B. Fy(a+b+)
    C. Fy(a–b+)
    D. Fy(a–b–)

93. Which of the following antibodies has been clearly implicated in transfusion reactions and hemolytic disease of the newborn?
    A. Anti-I
    B. Anti-K
    C. Anti-Le^a
    D. Anti-N

Refer to red cell panel chart 1 to answer questions 94–96.

RED CELL PANEL CHART 1

	Rh					MNSs				P	LEWIS		LUTHERAN		KELL					DUFFY		KIDD						
Cell #	D	C	E	c	e	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	IS	37	AHG	CkC
1	0	+	0	+	+	+	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	+	+	0	0	0	√
2	+	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	0	0	√
3	+	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	0	0	0	+	+	0	+	0	0	0	√
4	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	0	+	+	0	+	0	0	4+	
5	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	+	0	0	+	0	0	0	3+	
6	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	0	0	0	0	√
7	0	0	0	+	+	+	+	0	+	+	0	0	0	+	+	0	0	0	+	+	+	+	+	0	0	2+	
8	0	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	0	0	√
9	0	0	+	+	+	+	0	+	0	0	0	+	0	+	0	+	0	0	0	+	0	+	0	0	0	3+	
10	0	0	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	0	√
11	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	0	0	0	√
A/C																								0	0	0	√

94. Which of the following antibodies would require additional testing in order to be ruled out?
    A. Anti-E, -K, -Kp^a, -Js^a, -Jk^b
    B. Anti-E, -S, -Le^b, -K, -Kp^a, -Fy^a
    C. Anti-E, -S, -Le^a, -K, -Kp^a, -Js^a, -Fy^a, -Jk^a
    D. Anti-E, -Le^a, -K, -Kp^a, -Js^a, -Fy^b, -Jk^a, -Jk^b

95. The most likely antibody(ies) in the patient's serum is(are)
    A. Anti-S and anti-E
    B. Anti-E and anti-K
    C. Anti-Fy^b showing dosage
    D. Anti-K, anti-Js^a, and anti-Le^a

96. From the cells in red cell panel chart 2, choose a selected cell panel to help identify the antibody(ies) in the patient described in question 95.
    A. 1, 2, 5, 9, 10
    B. 2, 6, 7, 10
    C. 1, 4, 7
    D. 2, 3, 4, 6, 9

RED CELL PANEL CHART 2

	Rh					MNSs				P	LEWIS		LUTHERAN		KELL				DUFFY		KIDD		
Cell #	D	C	E	c	e	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a
1	0	+	0	+	+	+	+	+	0	0	+	0	0	+	0	+	0	0	0	+	0	+	+
2	+	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	0	+	+	+	+	+
3	+	+	0	0	+	0	+	0	+	+	0	+	0	0	0	+	0	0	0	+	+	0	+
4	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+
5	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	+	0	0	+	0
6	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	0
7	0	0	0	+	+	+	+	0	+	+	0	0	0	+	+	0	0	0	+	0	+	0	+
8	0	0	0	+	+	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	+	0
9	0	0	+	+	+	+	0	+	0	0	0	+	0	+	0	+	0	0	0	+	0	+	0
10	0	0	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0

97. Often when trying to identify a mixture of antibodies, it is useful to neutralize one of the known antibodies. Which one of the following antibodies is neutralizable?
    A. Anti-D
    B. Anti-Jk^a
    C. Anti-Le^a
    D. Anti-M

98. Which of the following antibodies does *not* match the others in terms of optimal reactive temperature?
    A. Anti-Fy^a
    B. Anti-Jk^b
    C. Anti-N
    D. Anti-U

99. A recently transfused patient's serum has a positive antibody screen. The panel performed at IS, in LISS at 37°C, and at AHG shows a strong anti-Fy^a and a weak possible anti-C. To confirm the anti-C, you would perform an
    A. Elution
    B. Absorption
    C. Antigen typing
    D. Enzyme panel

100. The antiglobulin test does not require washing or the addition of IgG-coated cells in which of the following antibody detection methods?
    A. Solid-phase red cell adherence assays
    B. Gel test
    C. Affinity column technology
    D. Polyethylene glycol (PEG) technique

101. Which set of antibodies could you possibly find in a patient with no history of transfusion or pregnancy?
    A. Anti-I, anti-s, anti-P_1
    B. Anti-Le^b, anti-A_1, anti-D
    C. Anti-M, anti-c, anti-B
    D. Anti-P_1, anti-Le^a, anti-I

102. Lymphocytotoxicity testing can be used to detect the presence of antibodies to
    A. Wr^a and Wr^b
    B. HLA antigens
    C. Bg^a, Bg^b, and Bg^c
    D. JMH antigen

103. In which of the following instances may mixed-field (mf) agglutination be observed?
    A. Direct antiglobulin test (DAT) result of patient undergoing delayed hemolytic transfusion reaction
    B. Indirect antiglobulin test (IAT) result of patient who has anti-Le^a
    C. DAT result of patient on high doses of α-methyldopa
    D. Typing result with anti-A of patient who is A_2 subgroup

104. The antibody produced during the secondary response to a foreign antigen is usually
    A. IgM
    B. A product of T lymphocytes
    C. Produced a month or more after the second stimulus
    D. Present at a higher titer than after a primary response

105. In which situation(s) may the ABO serum grouping *not* be valid?
    A. The patient has hypogammaglobulinemia.
    B. IgM alloantibodies are present.
    C. Cold autoantibodies are present.
    D. All the above

106. A group A, D-negative obstetric patient with anti-D (titer 256) is carrying a fetus who needs an intrauterine transfusion. Which of the following units should be chosen?
A. Group A, D-negative RBC
B. Group A, D-negative whole blood
C. Group O, D-negative RBC
D. Group O, D-negative whole blood

107. Which of the following is generally detected at the antiglobulin phase of testing?
A. Anti-Jk^a
B. Anti-M
C. Anti-P_1
D. Anti-I

108. Which of the blood group systems is associated with antibodies that are generally IgM?
A. Rh
B. Duffy
C. Kell
D. Lewis

109. Some antigens that are primarily found on white blood cells can occur on erythrocytes. Which of the following are the red blood cell equivalents of human leukocyte antigens (HLAs)?
A. Le^a, Le^b
B. Bg^a, Bg^b, Bg^c
C. Kp^a, Kp^b, Kp^c
D. Do^a, Do^b

110. The following phenotypes resulted from blood typing a mother, 6-month-old baby, and alleged father in a case of paternity testing.

	ABO	Rh	HLA
Mother	A	ce	A2, A29, B12, B17
Baby	O	ce	A2, A3, B12, B15
Alleged Father	A	DCce	A3, A9, B5, B27

Which of the following statements is true? The alleged father
A. Is excluded by the ABO system
B. Is excluded by the Rh system
C. Is excluded by the HLA system
D. Cannot be ruled out

INSTRUCTIONS: Each numbered group of incomplete statements (questions 111–123) is followed by four suggested responses. Select the *best* answer in each case. Lettered responses may be used once, more than once, or not at all.

Eight blood samples are received in the laboratory for ABO grouping. For each patient (questions 111–118), indicate the most likely cell and serum reactions selected from the lettered reaction matrix.

111. A patient with an acquired antigen due to infection with gram-negative bacteria

112. A patient with multiple myeloma

113. A newborn

114. An A_2 individual making an anti-A_1

115. A patient with antibodies to acriflavin (a yellow dye)

116. A patient who is immunodeficient

117. A patient with an unexpected IgM antibody in his serum

118. A patient with cold hemagglutinin disease (CHD)

	Cell Typing Results		Serum Typing Results		
	Anti-A	Anti-B	A_1 cells	B cells	O cells
A.	+	+	+	+	+
B.	+	0	+	+	0
C.	+	+	0	+	0
D.	0	+	0	0	0

For the following items (questions 119–123), select the answer that most closely corresponds to the description.

119. Found predominantly in whites

120. Associated with weak Kell system antigenic expression

121. Associated with the presence of chronic granulomatous disease

122. Linked with *MN*

123. A rare allele of *M* and *N*

A. McLeod phenotype
B. M^g
C. Kp^a
D. *Ss*

Antibody Identification, Transfusion Therapy, Transfusion Reactions

For questions 124–132, refer to red cell panel chart 3.

124. The racial origin of the donor of Cell #3 is most likely
A. Black
B. Eskimo
C. Oriental
D. White

125. The donor of Cell #5 is homozygous for which combination of the following genes?
A. *Ce*, P_1, *M*, *s*, *k*, Jk^a, Fy^a, Le^b
B. *Ce*, P_1, *s*, *k*, Jk^a, Fy^a, Le^b
C. *Ce*, *s*, *k*, Jk^a, Fy^a, Le^b, P_1
D. *Ce*, *s*, *k*, Jk^a, Fy^a

126. After testing a patient's serum with the panel, one observes there are no reactions at IS or 37°C with Cells #1–8. There is a 1+ AHG reaction with Cells #1 and #6 and a 3+ AHG reaction with Cells #4 and #5. All other Cells, #2, #3, #7, and #8, are negative at AHG. Which of the following statements is *true?*
A. Anti-Fy^a appears to be present.
B. Anti-Fy^a is present as well as an antibody that is reacting with an undetermined antigen on Cells #4 and #5.
C. Ficin will enhance the reactions of the antibody(ies) present.
D. Anti-Fy^a is present but can be ignored because most people are Fy(a–b–).

127. The serum of a patient tested with the reagent red cell panel using a low-ionic-strength-saline (LISS) additive demonstrates 3+ reactivity with Cells #1–8 at the antiglobulin phase. The autocontrol is negative. This pattern of reactivity is most likely due to
   A. Rouleaux formation
   B. Warm autoantibody
   C. Alloantibody directed against a high-frequency antigen
   D. Antibody directed against a preservative present in LISS

128. A patient's serum reacts with all the panel cells except Cell #7 at the antiglobulin phase only. Which of the following techniques would be most helpful at this point?
   A. Treat the panel cells with a proteolytic enzyme and repeat the panel with untreated serum.
   B. Treat the panel cells with dithiothreitol (DTT) and repeat the panel with untreated serum.
   C. Treat the patient's serum with dithiothreitol (DTT) and repeat the panel with treated serum.
   D. Treat the patient's serum with a proteolytic enzyme and repeat the panel with treated serum.

RED CELL PANEL CHART 3

Cell #	D	C	E	c	e	M	N	P_1	S	s	K	k	Jk^a	Jk^b	Fy^a	Fy^b	Lu^a	Le^a	Le^b	Xg^a
1	+	+	0	+	+	+	+	+	0	+	+	+	0	+	+	+	0	0	+	0
2	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+
3	+	+	0	+	+	+	0	+	+	0	0	+	+	+	0	0	0	+	0	+
4	+	0	+	+	0	+	+	0	+	0	0	+	+	0	+	0	0	0	+	0
5	0	+	0	0	+	+	+	+	0	+	0	+	+	0	+	0	0	0	+	0
6	0	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0	0	0
7	0	0	0	+	+	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+
8	0	0	0	+	+	+	+	0	+	+	0	+	+	0	0	+	0	+	0	+

In addition to red cell panel chart 3, use the following information to answer questions 129–132.

The patient is group A, D-negative and has not been recently transfused. Cells #5, #6, and #7 are negative in all phases with this patient's serum. The autocontrol is negative. Other cell results are as follows:

Cell #	IS	37°C LISS	AHG
1	0	1+	4+
2	0	1+	4+
3	2+	1+	4+
4	0	1+	4+
8	2+	0	0

129. From the reactions given, it appears that there is(are)
   A. One antibody reacting
   B. One antibody reacting that shows dosage
   C. "Cold" and "warm" antibodies reacting
   D. Two "warm" antibodies reacting

130. The antibody that reacts at immediate spin is most likely
   A. Anti-D
   B. Anti-P_1
   C. Anti-Le^a
   D. Anti-Le^b

131. The antibody that reacts at 37°C and with AHG is
   A. Anti-C
   B. Anti-D
   C. Anti-CD
   D. Anti-K

132. What should you do to increase the probability that an antibody identification is correct?
   A. Make an eluate.
   B. Do saliva testing.
   C. Run an additional panel.
   D. Type the patient's cells for the corresponding antigens.

133. The following results were obtained upon testing a specimen of a patient, being admitted after a car accident, who had no recent history of transfusion or medical problems.
   ABO group: A
   Rh type: D-positive
   Antibody screening test: Positive, one screening cell only
   Direct antiglobulin test: Negative
   Antibody identification: Anti-K identified; 3 K+ cells that reacted with the patient serum and 3 K– cells that did not react with the patient serum were on the panel.
   Patient's cell phenotyping: K+

   What is the most likely cause of the discrepant results?
   A. Failure to read panel at antiglobulin phase
   B. Failure to use positive and negative controls with anti-K
   C. Panel cell reactions interpreted incorrectly
   D. Patient has circulating donor cells that are K+

134. False negative results at the antiglobulin phase of an antibody screening test are most likely due to
   A. Excessive washing of the red cells
   B. Inadequate washing of the red cells
   C. Warm autoantibody present in the patient's serum
   D. Failure to allow the blood to clot properly

135. What is the process of removing an antibody from the red blood cell membrane called?
A. Absorption
B. Adsorption
C. Elution
D. Immunization

136. At the end of an antiglobulin test, IgG-coated control cells are added to the negative tests and centrifuged. What does it mean if no agglutination occurs?
A. Test is valid.
B. Antiglobulin reagent was working properly.
C. Cells were not washed thoroughly.
D. Control cells are contaminated.

137. The crossmatch is performed using
A. Donor's serum and recipient's red cells
B. Donor's red cells and recipient's serum
C. Donor's serum and reagent red cells
D. Recipient's serum and reagent red cells

138. A male trauma victim whose blood type is group AB, D-negative has a negative antibody screening test. He has been transfused with both of the group AB, D-negative units in inventory within the last hour. He is now in surgery and expected to need large amounts of blood. Of the following available units in inventory, which type should be given next?
A. 30 units of group O, D-positive
B. 26 units of group A, D-positive
C. 10 units of group O, D-negative
D. 5 units of group A, D-negative

139. Which of the following will the crossmatch do?
A. Prevent immunization
B. Prevent delayed transfusion reactions
C. Guarantee normal survival of the red blood cells
D. Frequently verify donor ABO compatibility

140. Given that a patient's antibody screening test is negative, which of the following may cause a false positive result in a compatibility test?
A. Incorrect ABO typing of the donor or patient
B. An alloantibody against a low-frequency antigen on the donor cells
C. Prior coating of IgG antibody on the donor cells
D. All the above

141. Which of the following will be incompatible in the crossmatch?

	Donor	Recipient
A.	Group A, D-negative	Group A, D-positive
B.	Group O, D-positive	Group A, D-positive
C.	Group AB, D-positive	Group A, D-positive
D.	Group A, D-positive	Group A, D-negative

142. A resident physician hand-delivers a blood sample, drawn by the attending physician, for pretransfusion testing from a patient who is difficult to draw. The sample is unlabeled. One should
   A. Discard the sample and request that the resident obtain a new sample, adhering to proper guidelines for labeling
   B. Label the specimen with the information the resident provides
   C. Label the specimen with information from the accompanying transfusion request form
   D. Request the sample be returned to the nursing station to be labeled

143. A specimen of blood is received in the blood bank with request slips initialed by the phlebotomist. The tube has the patient's first and last name and medical records identification number on the label. What else must be on the tube label as required by AABB *Standards?*
   A. Patient's room number
   B. Date of phlebotomy
   C. Initials of phlebotomist
   D. Attending physician's name

144. A physician calls the blood bank and wants an additional unit of RBC crossmatched for a patient. Several specimens from that patient are identified that have been drawn over the past month. Which of the following available samples is the oldest acceptable specimen that may be used for crossmatching?
   A. 1 day old
   B. 4 days old
   C. 1 week old
   D. 1 month old

145. A patient has a hemoglobin value of 8.1 g/dL. The surgeon wants to raise the hemoglobin to 10 g/dL before surgery. How many units of RBC need to be administered to this patient to raise the hemoglobin to the required level?
   A. 1
   B. 2
   C. 3
   D. 4

146. A patient with an anti-K and an anti-Jk^a in her plasma needs 2 units of RBC for surgery. How many group-specific units would need to be screened to find 2 units of RBC? The frequency of Jk(a+) is 77%; the K+ frequency is 10%.
   A. 6
   B. 10
   C. 20
   D. 36

Use the following information to answer questions 147 and 148.

A postpartum female is bleeding because of disseminated intravascular coagulation (DIC). The attending physician orders cryoprecipitate for fibrinogen replacement. The freezer inventory contains the following cryoprecipitate: 6 bags Group A, 8 bags Group O, 6 bags Group AB, 12 bags Group B.

147. How many bags (units) should be thawed and pooled to provide 2 g of fibrinogen?
   A. 2
   B. 4
   C. 8
   D. 10

148. The patient is group A. Which cryoprecipitate units would most appropriately be used to treat this patient?
A. Group A only
B. Group AB only
C. Group A and Group O
D. Group A and Group AB

149. If 98% of the red blood cells are viable in a unit of RBC at the time of transfusion, what percentage of red cells will remain viable 28 days posttransfusion?
A. 10%
B. 30%
C. 50%
D. 70%

150. What is the component of choice for someone who needs a RBC transfusion when there is a history of febrile transfusion reactions?
A. RBCs less than 5 days old
B. Leukocyte-reduced RBCs
C. RBCs 30 to 35 days old
D. Frozen RBCs that have been thawed and deglycerolized

151. Which of the following is the component of choice when a physician is concerned about restoring or maintaining oxygen-carrying capacity?
A. Albumin
B. Cryoprecipitate
C. Whole blood
D. Red blood cells

152. The serum of a patient contains an antibody that reacts with all random donor cells and panel cells that have been tested. The best possibility to find compatible blood would be to test
A. Grandparents
B. Parents
C. Siblings
D. Spouse

Use the following information to answer questions 153 and 154.

A resident physician on the trauma team runs a pretransfusion blood sample from a male trauma victim to the blood bank and wants 6 units of blood to be issued immediately. He indicates that he is willing to sign for uncrossmatched blood. He also indicates that he wants 6 units ready at all times. The patient has been admitted to this institution previously for GI bleed.

153. The resident says the victim has a donor card in his wallet indicating a group B, D-positive blood type. What should be done immediately?
A. Issue 6 units of uncrossmatched group B, D-positive whole blood.
B. Check patient and donor records to confirm the blood type, then issue 6 units of uncrossmatched group B, D-positive blood.
C. Withhold blood until ABO and compatibility testing are completed.
D. Issue 6 units of uncrossmatched group O RBCs.

154. What should be the next step in the work-up of this emergency department patient?
A. Prepare 6 units uncrossmatched group B, D-positive whole blood.
B. Check blood bank records for any previous patient information.
C. Type and screen the patient sample.
D. Prepare 6 more units of uncrossmatched group O blood.

155. Four units of fresh-frozen plasma have been ordered to correct factor V deficiency in a group O patient. One should thaw and issue _____ plasma.
   A. Group O only
   B. Group O and/or group A
   C. Group O and/or group AB
   D. Any blood group available

156. Which of the following is acceptable to be given intravenously with a blood transfusion?
   A. 5% dextrose in water
   B. Physiologic saline
   C. Ringer's solution
   D. Potassium chloride in saline

157. Hemolytic transfusion reactions are the most serious type of reactions to blood transfusion. The majority of hemolytic transfusion reactions are caused by _____ errors.
   A. Blood typing
   B. Antibody identification
   C. Clerical
   D. Crossmatching

158. What type of transfusion reaction is often diagnosed by a positive DAT and a gradual drop in the patient's hemoglobin level?
   A. Anaphylactic
   B. Febrile
   C. Delayed hemolytic
   D. Acute hemolytic

159. What antibody, labile both in stored serum and the patient's plasma, is a frequent cause of delayed hemolytic transfusion reactions?
   A. Anti-A
   B. Anti-D
   C. Anti-Jk^a
   D. Anti-K

160. Occasionally, patients have an anaphylactic reaction to a specific immunoglobulin class during a transfusion. Which immunoglobulin class is most often implicated?
   A. IgA
   B. IgD
   C. IgE
   D. IgG

161. A transfusion of which of the following is *least* likely to transmit HIV, HCV, or HBV?
   A. Pooled plasma, solvent/detergent treated
   B. Cryoprecipitate
   C. Leukocyte-reduced RBCs
   D. Platelets

Use the following information to answer questions 162–164.

A transfusion reaction is reported by the nursing unit on patient A. D. The nurse reports that the patient had chills, fever, and back pain within a few minutes of starting the unit. The nurse asks what s/he should do.

162. You tell the nurse to immediately
   A. Collect posttransfusion blood samples
   B. Monitor the pulse and blood pressure
   C. Discontinue the unit, keep the line open
   D. Page the patient's physician for instructions

163. Which of the following directives would *not* be included in the additional activities you would request the nurse to do?
A. Return the unit to the blood bank.
B. Obtain a posttransfusion blood sample in EDTA.
C. Obtain a posttransfusion urine sample.
D. Obtain a fresh unit from the blood bank for immediate infusion.

164. All paperwork checks on this transfusion reaction are OK. The pretransfusion sample has straw-colored plasma. The posttransfusion sample has red-tinged plasma. This is indicative of a(an)
A. Uncomplicated transfusion
B. Intravascular transfusion reaction
C. Error in which drugs have been infused with the blood
D. Febrile transfusion reaction

165. Although use of autologous blood transfusions generally has several advantages, which of the following is *not* avoidable?
A. Transmission of disease
B. Clerical error
C. Allergic reactions
D. Graft-versus-host disease

166. Before blood is issued for transfusion, a patient's previous blood bank records must be reviewed. Which of the following is *not* included in this review process?
A. ABO group and Rh type
B. Clinically significant antibodies
C. Serious adverse reactions
D. Hepatitis testing

167. Which of the following would *not* cause a positive hemagglutination reaction in the crossmatch?
A. Incorrect ABO grouping of the donor
B. Unexpected antibodies in the recipient serum
C. A positive DAT on the recipient red cells
D. A positive DAT on the donor red cells

168. Which of the following blood types necessitates that a separate Rh control tube be set up when using a monoclonal anti-D reagent?
A. Group O, D-positive
B. Group A, D-positive
C. Group B, D-positive
D. Group AB, D-positive

169. Six units of blood were ordered stat for a young female patient who has the following tube typing results (the tube typing procedure uses a washed red cell suspension with monoclonal reagents). The physician has just called requesting emergency release of 2 units of RBCs.

Cell Typing Results				Serum Typing Results	
Anti-A	Anti-B	Anti-D	Rh control	A_1 cells	B cells
2+	4+	3+	2+	4+	4+

Which of the following should be done first?
A. Perform a DAT on the patient's red cells.
B. Tell the physician that no blood can be released until a full work-up has been done.
C. Begin the antibody screening test.
D. Select 2 units of group O, D-negative RBCs for emergency release.

170. Referring to the tube typing results in question 169, the most probable cause of the patient's positive Rh control test is that the patient has
   A. A positive DAT result with anti-IgG
   B. A cold autoantibody
   C. Leukemia
   D. Multiple myeloma

171. A patient experiences severe rigors and goes into shock after receiving part of a unit of RBC. The patient's temperature, which was 37.5°C pretransfusion, is now 40.0°C. Which of the following is the most likely type of reaction?
   A. Hemolytic
   B. Anaphylactic
   C. Septic
   D. Embolic

172. Referring to the reaction described in question 171, the incidence of this type of reaction is highest with which of the following components?
   A. RBC
   B. FFP
   C. Cryoprecipitate
   D. Platelets

173. The serum of a patient transfused 2 weeks ago reacts 3+ on immediate spin and 1+ at the antiglobulin phase of testing with all reagent red cells except for the *ii* cell. The autocontrol reacts similarly to the panel cells. In order to crossmatch this patient, one should
   A. Use autoadsorbed serum
   B. Use the prewarmed technique
   C. Identify the antibody and obtain blood from the rare donor file
   D. Use a LISS additive

INSTRUCTIONS: Each numbered set of test results or conditions (questions 174–184) is followed by four or five lettered responses. Select the *best* answer in each case. Lettered responses may be used once, more than once, or not at all.

Six units of blood from volunteer donors are tested for ABO group, Rh type, and unexpected antibodies. For each set of test results (questions 174–179), indicate the final disposition of the donated unit. Assume additional FDA required testing is nonreactive, unless noted.

	Cell Typing Results			Serum Typing Results		
	Anti-A	Anti-B	Anti-D	A_1 cells	A_2 cells	B cells
174.	0	0	3+	4+	3+	4+
175.	0	0	0	4+	4+	4+
	Weak D test = 3+, DAT = 0					
176.	0	0	0	4+	4+	4+
	Weak D test = 1+, DAT = 1+					
177.	0	0	3+	2+	0	4+
178.	0	0	3+	0	0	4+
179.	0	0	3+	4+	4+	4+
	Antibody screen = positive Antibody identification = anti-Fy^a					

   A. Label group O, D-positive
   B. Label group O, D-negative
   C. Label the RBC group O, D-positive; do not use the plasma
   D. Perform additional testing
   E. Discard the unit

For the following conditions (questions 180–184), select the blood component of choice for treatment.

180. von Willebrand disease

181. Hypofibrinogenemia

182. Factor V deficiency

183. Liver disease

184. Hemorrhagic episode during intensive chemotherapy

A. Platelet concentrate
B. RBC
C. Cryoprecipitate
D. Fresh-frozen plasma

Hemolytic Disease (Hemolytic Disease of the Newborn, Immune Hemolytic Anemia)

185. Which of the following would *not* be included when routine testing is performed early in a pregnancy?

A. ABO and Rh testing
B. Antibody screening
C. Amniocentesis
D. Weak D testing on apparent Rh-negative patients

186. In which of the following blood group systems may the red blood cell typing change during pregnancy?

A. P
B. MNS
C. Lewis
D. Duffy

187. Which of the following is *not* considered a useful predictor of hemolytic disease of the newborn (HDN) during the gestational period?

A. Anti-A
B. Anti-D
C. Anti-Fy^a
D. Anti-U

188. Which is the class of immunoglobulin uniquely associated with hemolytic disease of the newborn (HDN)?

A. IgA
B. IgD
C. IgE
D. IgG

189. A neonate with a positive direct antiglobulin test (DAT) indicates that there was an incompatibility between a mother and her fetus. The system that is most commonly associated with an incompatibility is

A. ABO
B. Rh
C. Kell
D. Kidd

190. The cord blood of an infant of a D-negative mother with anti-D, titer 2048, is submitted to the laboratory along with a sample of maternal blood with a request to select blood for possible exchange transfusion. The neonate appears to be D-negative. The weak D status cannot be determined because the DAT result is positive (4+). What is the most likely explanation for this?

A. Wharton's jelly contaminated the sample.
B. The baby has ABO HDN.
C. The baby has a "blocked D" antigen.
D. A different antibody is causing the positive DAT.

191. A newborn is group O, D-positive and has a 3+ DAT. The mother's antibody screening test is negative. Assuming the antibody detection test is valid, one should consider HDN due to an antibody directed against

A. Fy^b antigen
B. K antigen
C. Low-incidence antigen
D. A or B antigen

192. The most conclusive way to demonstrate the antibody that is causing a positive DAT in a newborn is to perform an antibody
A. Titration using the mother's serum
B. Panel using the mother's serum
C. Panel using an eluate from the mother's red cells
D. Panel using an eluate from the baby's red cells

193. Which two of the following conditions are the most serious immediate consequences of HDN?
A. Anemia and a positive DAT
B. Hyperbilirubinemia and anemia
C. Hyperbilirubinemia and jaundice
D. Hyperbilirubinemia and kernicterus

194. A premature infant with *hydrops fetalis* and a bilirubin of 20 mg/dL is referred to an intensive care unit. The neonatologist wants to perform an exchange transfusion to correct anemia and prevent kernicterus. No blood specimen from the mother is available. The infant's serum has a positive antibody screen. The DAT is 4+. What would be the best approach in this situation?
A. Identify the antibody in the serum and crossmatch blood negative for the offending antigen, using the serum in a crossmatch.
B. Issue group O, D-negative blood for the exchange.
C. Refuse to issue blood for exchange until a sample can be obtained from the mother.
D. Identify the antibody in the serum and eluate and crossmatch blood negative for the offending antigen, using both the serum and eluate in a crossmatch.

195. Which of the following is *not* true of an exchange transfusion when an infant is suffering from HDN?
A. Removes unconjugated bilirubin
B. Reduces the amount of incompatible antibody in the baby's circulation
C. Removes antibody-coated red blood cells
D. Provides red blood cells of the baby's type

196. A massive fetomaternal hemorrhage in a D-negative woman who had a D-positive infant should be suspected if the
A. Infant is premature
B. Infant has a positive acid elution slide test
C. Mother requires a transfusion following childbirth
D. Weak D test on the maternal blood shows a mixed-field reaction microscopically

197. A D-negative woman who received antepartum RhIG gave birth to a D-positive infant and received one vial of RhIG the same day. Because of postpartum hemorrhage, her physician ordered two units of RBCs for her 2 days later. The antibody screening test was positive, but the crossmatches were both compatible. The most likely cause for the positive antibody screening test was the presence of a(an)
A. Clinically significant anti-K
B. Actively acquired anti-D
C. Passively acquired anti-D
D. Rh antibody other than anti-D

198. What is the principle of the Kleihauer-Betke stain?
   A. Fetal hemoglobin is more resistant to alkaline buffer than adult hemoglobin.
   B. Adult hemoglobin is more resistant to alkaline buffer than fetal hemoglobin.
   C. Fetal hemoglobin is more resistant to erythrosin and hematoxylin staining than adult hemoglobin.
   D. Adult hemoglobin is more soluble in acid buffer than fetal hemoglobin.

199. Which of the following antibodies present in a multitransfused obstetric patient would be most likely to cause HDN in her infant?
   A. Anti-Le^a
   B. Anti-c
   C. Anti-P_1
   D. Anti-K

Use the following information to answer questions 200 and 201.

A Kleihauer-Betke acid elution stain for postpartum fetomaternal hemorrhage (FMH) is reported to be 1.3%.

200. What is the total volume of FMH?
   A. 6.5 mL
   B. 13 mL
   C. 26 mL
   D. 65 mL

201. With this amount of FMH, how many vials of a standard dose of RhIG should be administered to the mother within 72 hours of childbirth? (Presume the infant to be D-positive.)
   A. 1
   B. 2
   C. 3
   D. 4

Use the following information to answer questions 202–205.

A 64-year-old female is seen in the emergency department with a hemoglobin value of 8.9 g/dL. The resident sends down a request for 2 units of packed red cells. She types as group O, D-positive using monoclonal antisera. Her ABO group and Rh type match previous records. She has not been transfused in the past 5 years. However, her antibody screen produces the following results:

	Screen Cell I	Screen Cell II	Screen Cell III	Auto-control
IS	0	0	0	0
37°C LISS	0	0	0	0
AHG	4+	4+	4+	4+
CC	NT*	NT	NT	NT

*NT = not tested

202. What is the most likely cause for these results?
   A. Polyagglutination
   B. Rouleaux
   C. Transfusion reaction
   D. Warm autoantibody

203. To demonstrate whether the antibody(ies) has/have become attached this patient's red blood cells *in vivo*, which of the following tests would be most useful?
   A. Direct antiglobulin test
   B. Complement fixation test
   C. Elution procedure
   D. Indirect antiglobulin test

204. How would you identify the antibody(ies) on this woman's cells?
   A. Autoabsorption followed by a panel on the absorbed serum
   B. Elution followed by a panel on the eluate
   C. Enzyme-treated panel on her serum
   D. Perform a panel on the serum.

205. What is the best treatment for this woman's anemia?
   A. Transfusion with packed red cells
   B. Infusion of fresh-frozen plasma
   C. Steroid administration
   D. Plasma exchange

206. The specificity of the antibody in warm autoimmune hemolytic anemia (WAIHA) is most often associated with which of the following blood group systems?
   A. ABO
   B. Kell
   C. Kidd
   D. Rh

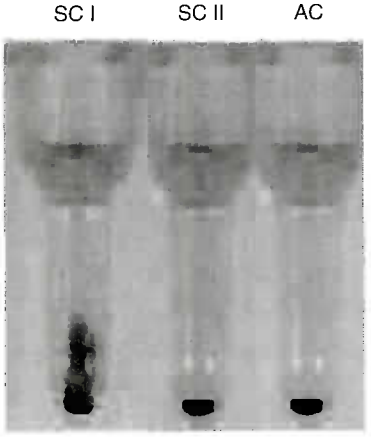
207. What is the most important consideration in patients suffering from life-threatening anemia and whose serum contains warm autoantibodies?
   A. Determine the specificity of the autoantibody.
   B. Determine the immunoglobulin class of the autoantibody.
   C. Exclude the presence of alloantibody(ies).
   D. Avoid transfusion.

208. The serum and eluate from a male patient with a 3+ DAT on α-methyldopa therapy demonstrates anti-e specificity. The patient denies knowledge of having received blood transfusions. To determine whether the anti-e is an auto- or alloantibody, one should
   A. Type the patient's red cells with a low-protein anti-e reagent
   B. Adsorb the serum with the patient's red cells
   C. Adsorb the eluate with R_2R_2 red cells
   D. Adsorb the eluate with rr red cells

209. A patient has a 2+mf DAT with anti-IgG. He was transfused 1 week ago with 2 units of RBCs during surgery. His eluate would most likely contain
   A. No antibody
   B. Autoantibody
   C. Alloantibody
   D. Drug-related antibody

Use the following information to answer questions 210 and 211.

An antibody screen was performed on a 25-year-old male referred to the hospital for elective surgery. Refer to the gel reactions below seen in an antibody screen test. (SC = screening cell, AC = autocontrol)



210. Which of the following is the correct interpretation of the reaction in SC I?
   A. 0
   B. 1+
   C. 2+
   D. 3+

211. What has happened to the matrix in SC I that caused a difference in its appearance from that of SC II?
   A. Antibody-coated cells have been trapped in the gel matrix of SC I.
   B. A too heavy red cell suspension was used in SC I; correct suspensions were used in SC II and AC.
   C. Hemolysis is occurring in SC I, but not in SC II or AC.
   D. SC II shows a mixed-field reaction.

212. How is cold hemagglutinin disease (CHD) different from paroxysmal cold hemoglobinuria (PCH)?
   A. PCH is a common form of cold autoimmune anemia whereas CHD is rare.
   B. PCH is a warm autoimmune hemolytic anemia.
   C. The offending antibody in PCH is an IgG antibody unlike the IgM antibody in CHD.
   D. The offending antibody in PCH is an IgM antibody while an IgG antibody is common in CHD.

213. If during a Donath-Landsteiner test there is hemolysis in both the test and control tubes at the conclusion of the test, this indicates that the test is
   A. Positive
   B. Negative
   C. Invalid
   D. False negative

214. A patient has a positive DAT due to cephalosporin therapy and a negative antibody screening test result. Two units of RBCs have been ordered. In order to crossmatch this patient, one should crossmatch with
   A. The eluate from the patient's red cells and donor cells
   B. Autoadsorbed patient's serum and untreated donor cells
   C. Untreated patient's serum and untreated donor cells
   D. Cephalosporin-treated donor cells and untreated patient's serum

215. A patient being treated with α-methyldopa has a 4+ DAT result. You would expect an eluate from his red cells to react most likely with
   A. All the untreated panel cells tested
   B. Just the untreated D-positive cells tested
   C. All panel cells treated with α-methyldopa
   D. All panel cells when α-methyldopa is added to the eluate

216. A patient with drug-induced hemolytic anemia has the following DAT results:
   Polyspecific AHG = 3+
   Anti-IgG = 3+
   Anti-C3d = 0
   Which of the following drugs is most likely to be the cause?
   A. Phenacetin
   B. Quinidine
   C. Penicillin
   D. Tolmetin

217. A patient with cold hemagglutinin disease (CHD) has a positive DAT when tested with a polyspecific AHG. Which of the following would most likely be detected on her red cells?
   A. IgM
   B. IgG
   C. IgA
   D. C3

218. If a patient's red blood cells are DAT+ due to penicillin antibody, the
   A. Serum will react if penicillin is added to the test system
   B. Serum will react with all red cells
   C. Eluate will react with penicillin-coated red cells
   D. Eluate will react with all red cells

219. A patient's preoperative antibody screening test is negative, but the autocontrol is positive. A DAT performed on his red cells is 2+ with anti-IgG. His last transfusion was 9 months ago, and he has a negative drug history. Which of the following would most likely be present in his eluate?
   A. No antibody
   B. Alloantibody
   C. Alloantibody and autoantibody
   D. Autoantibody

220. A patient with WAIHA has a history of an anti-Jk^a in her autoabsorbed serum, and an anti-e in her eluate. Her autoabsorbed serum today is not showing anti-Jk^a on prewarmed panel, but the eluate is still showing anti-e. What blood would be selected for cross-matching packed red cells today?
   A. e-Negative
   B. Jk(a–)
   C. Jk(a–) and e-negative
   D. No screening is necessary because all transfused cells will be destroyed anyway.

INSTRUCTIONS: The numbered group of incomplete statements (questions 221–228) is followed by four suggested responses. Select the *best* answer in each case. Lettered responses may be used once, more than once, or not at all.

For the following situations (questions 221–228), indicate whether the women are candidates for Rh immune globulin (RhIG) prophylaxis. Assume that D-negative mothers have a negative test for weak D and a nonreactive antibody screening test unless noted.

221. Mother D-negative; infant weak D

222. Mother weak D (strong); infant D-positive

223. Mother D-negative; twin #1 D-negative, twin #2 D-positive

224. Mother D-negative with anti-Fy^a; infant D-positive

225. Mother group O, D-negative; infant group A, DAT = 2+, monoclonal anti-D-negative at immediate spin, weak D test not performed

226. Mother D-negative, with anti-D, titer 2, history of RhIG injection postamniocentesis procedure at 30 weeks; infant D-positive

227. Female D-negative; miscarriage at 11 weeks

228. Mother D-negative; infant D-positive; rosette test = 1−2 rosettes per field

A. Yes, 50-μg dose
B. Yes, 300-μg dose
C. Yes, additional testing necessary to determine dose.
D. RhIG is not indicated.

answers & rationales

Blood Collection, Preservation, Processing, Component Preparation, and Quality Control

1.

C. Some potential donors are rejected to protect the recipient, and others are rejected to protect themselves. In this case, the woman meets the criteria except that her hematocrit is too low, and the loss of a unit of blood may have a detrimental effect on her. The minimum acceptable hematocrit is 38%.

2.

C. Donors are allowed to donate no more than 10.5 mL/kg of their body weight. This amount includes the samples used for testing drawn at the time of collection. The calculation for a 95-lb donor is

$95 \text{ lb} \div 2.2 \text{ lb/kg} = 43.2 \text{ kg}$

$43.2 \text{ kg} \times 10.5 \text{ mL/kg} = 453.6 \text{ mL}$

If less than 300 mL is to be collected, the anticoagulant must be reduced proportionately.

3.

A. Hepatitis viruses and HIV have extended incubation periods in which exposure has occurred but neither serological nor clinical manifestations of the disease are evident. The current screening tests, although quite sensitive, are unable to detect the viruses if testing is performed during this incubation period. To safeguard against the possibility that the donor received blood or blood products collected during the incubation period, a 12-month deferral is incurred to allow for fulmination of the disease.

4.

D. During autologous presurgical donation, a different set of criteria is used for donor acceptability. All the conditions listed are acceptable with the exception of the bacteremia. The bacteria may proliferate in the stored blood and be reinfused into the donor (patient) during or after the surgery. Even treatment for a suspected bacteremia is a contraindication for autologous donation.

5.

D. Recipients of human growth hormone are deferred indefinitely because of the risk of transmission of Creutzfeldt-Jakob disease. Recipients of recombinant growth hormone incur no deferral. A history of either syphilis or gonorrhea causes a deferral of 12 months from completion of treatment. Accutane®, a drug used to treat acne, may be a teratogen and requires a 1-month deferral after receipt of the last dose.

6.

A. Of the viruses listed, CMV is the only one that resides exclusively in leukocytes. Although CMV transmission is not a problem for most patients, it can cause serious disease in low-birth-weight neonates of CMV-negative mothers and immunocompromised patients. These patients should be transfused with CMV seronegative or leukocyte-reduced cellular components.

7.

C. Donors who have ingested aspirin within 36 hours of donation need not be excluded for whole blood donation. The platelets prepared from such donors should be labeled and may be used in a multiple pool prepared for adult transfusion. Because aspirin affects platelet function, a single unit of platelet concentrate from this donor should not be used for platelet therapy for infants and neonates. This donor should not be the sole source of platelets and, therefore, would be temporarily deferred as a plateletpheresis donor.

8.

C. Under no circumstances should any blood component from a high-risk donor be released from the donor center to a transfusion unit. Donors in high-risk groups for AIDS must be deferred from donating. If high-risk activity becomes known retrospective to blood donation (such as in the self-exclusion process), the blood components from the donation must be retrieved and destroyed.

9.

C. Plasma protein fraction (PPF) and albumin preparations (5% and 20%) provide colloid replacement and volume expansion with virtually no risk of viral transmission. These are pooled products and are pasteurized by heating to 60°C for 10 hours. Other products, such as clotting factor concentrates, are usually treated by solvent-detergent method to inactivate viruses with lipid envelopes such as HBV, HCV, HIV, and HTLV-I.

10.

B. It is acceptable according to FDA and AABB *Standards* to screen donors for infectious diseases in pools of 16 to 24 donor sera. If a donor pool is positive for HCV, all individual donors making up the pool are tested individually using the same nucleic acid test (NAT) to find the positive donor. When that donor is identified, s/he is excluded from donating henceforth and all components from that donation are retrieved and destroyed.

11.

D. Cryoprecipitate provides the only known concentrated source of fibronectin, useful in the phagocytic removal of bacteria and aggregates by the reticuloendothelial system. It also contains factors VIII:C, VIII:vW, and XIII. Antithrombin III (AT III), necessary to prevent a thromboembolic disorder, is depleted in DIC and liver disease. Transfusion sources of AT III are fresh-frozen plasma (FFP) and commercial concentrates, but AT III is not present in cryoprecipitate.

12.

D. FFP contains all the plasma clotting factors. FFP's primary use is for patients with clotting factor deficiencies for which no concentrate is available and patients who present multiple factor deficiencies such as in liver disease. Platelets are cellular elements, not a plasma clotting factor, and they must be maintained at 20–24°C with continuous gentle agitation to maintain their viability.

13.

C. The AABB *Standards* require that when a patient is likely at risk for graft-versus-host disease (GVHD), all cellular blood components must be irradiated before transfusion. This includes components for patients who are immunodeficient or immunoincompetent, such as a patient on immunosuppressive therapy and a fetus who receives intrauterine transfusion. Irradiation of RBCs for exchange transfusion is not required by AABB *Standards,* although many hospital transfusion services do so. Immunocompetent individuals require irradiated components if they are to receive cellular components from someone who may be homozygous for a shared HLA haplotype, such as a blood relative or an HLA-matched donor. Gamma irradiation of cellular components is the *only* way to prevent transfusion-associated GVHD that occurs when immunocompetent donor T cells survive in the patient's circulation and mount an immune response against the host cells. A minimum of 25 Gy delivered to the midplane of the container and at least 15 Gy to all other areas will prevent GVHD.

14.

A. The limiting criterion for *in vitro* storage of blood is the survival in the recipient of at least 75% of the transfused red cells for at least 24 hours after transfusion. Additional adenine in an anticoagulant-preservative formulation provides a substrate for the continued generation of ATP *in vitro*. The overall effect is improved viability.

15.

D. Donor blood may not be labeled according to test results obtained from previous donations. Several segments removed from the donor unit will provide sufficient sample for all required testing but will limit the number of segments available for crossmatching. After centrifugation, the plasma may be removed from the segments and clotted with calcium chloride or a similar commercial product for use in test procedures requiring serum. Alternatively, institutions with sterile connecting devices may attach a small bag and remove an aliquot sufficient for testing.

16–17.

(16:C, 17:A) *In vitro* recovery of factor VIII must be assayed monthly to ensure proper control of conditions during cryoprecipitate production. A minimum of 80 international units (IU) per container must be present in the final product. One international unit is defined as the clotting activity of 1 mL of fresh plasma. The total number of factor VIII units is calculated from the formula:

$$\text{Factor VIII (IU/mL)} \times \text{volume (mL)} = \text{Total IU Factor VIII}$$

In this case, 9 IU/mL × 10 mL = 90 IU per container in the final product. This exceeds the required 80 IU/container and so meets AABB *Standards*.

Although there is no existing standard for percent recovery of factor VIII during production, this information may be helpful in monitoring various stages of production when the monthly quality control assays fall below the acceptable standard. Recovery can be calculated by the formula:

$$\frac{\text{Post (F VIII IU/mL} \times \text{volume mL)}}{\text{Pre (F VIII IU/mL} \times \text{volume mL)}} \times 100 = \%\ \text{Factor VIII recovery}$$

In this instance,

$$\frac{9\ \text{IU/mL} \times 10\ \text{mL}}{1\ \text{IU/mL} \times 250\ \text{mL}} \times 100 = 36\%\ \text{Factor VIII recovery}$$

18–21.

(18:D, 19:D, 20:B, 21:B) Each centrifuge used for platelet production must be calibrated upon receipt or after major repair. Calculating percent recovery during various stages of platelet production is a valuable troubleshooting tool when monthly quality control assays consistently fall below the minimum standard of 5.5×10^{10} platelets per platelet concentrate. In this exercise, the first spin gives an 83% platelet yield, and the second spin produces a 60% yield from the PRP. Increasing the time or rpm of the PRP will result in a greater percent yield for the platelet concentrate. Yield is calculated from the following formulas:

$$\text{Platelet count}/\mu\text{L} \times 1000\ \mu\text{L/mL} \times \text{Volume (mL)} = \text{Total count in component}$$

$$\frac{\text{Total count in PRP}}{\text{Total count in whole blood}} \times 100 = \%\ \text{yield from whole blood}$$

$$\frac{\text{Total count in platelet concentrate}}{\text{Total count in PRP}} \times 100 = \%\ \text{yield from PRP}$$

In the example above, the total count in PRP would be

$$300{,}000/\mu\text{L} \times 1000\ \mu\text{L/mL} \times 250\ \text{mL} = 7.5 \times 10^{10}\ \text{platelets}$$

The total count in whole blood is

$$200{,}000/\mu\text{L} \times 1000\ \mu\text{L/mL} \times 450\ \text{mL} = 9 \times 10^{10}\ \text{platelets}$$

The percent yield from whole blood is

$$\frac{7.5 \times 10^{10}}{9 \times 10^{10}} \times 100 = 83\%$$

The total count in the platelet concentrate is

$$900{,}000/\mu\text{L} \times 1000\ \mu\text{L/mL} \times 50\ \text{mL} = 4.5 \times 10^{10}\ \text{platelets}$$

The percent yield from PRP is

$$\frac{4.5 \times 10^{10}}{7.5 \times 10^{10}} \times 100 = 60\%$$

At least 75% of prepared platelet packs must contain a minimum of 5.5×10^{10} cells. Because there are only 4.5×10^{10} cells in this platelet concentrate, the platelet yield would be considered too low.

22.

B. A low red blood cell concentration of 2,3-BPG increases red cell affinity for O_2, causing less O_2 to be released to the tissues. As blood is stored, 2,3-BPG levels fall. Once the blood is transfused, red cells regenerate 2,3-BPG and ATP, which are fully restored in about 24 hours. Other metabolic changes that occur as blood is stored are an increase in plasma K^+ as red cells leak K^+, an increase in plasma hemoglobin, and a decrease in ATP.

23.

D. Autologous blood should not be drawn later than 72 hours prior to surgery. The reason is to allow time for adequate volume repletion. However, the medical director may decrease this time if the patient's condition warrants it.

24.

B. Preoperative autologous donation is commonly done for orthopedic surgery, radical prostatectomy, and open heart surgery. Patients with uncompensated anemia and hemoglobin levels below 11.0 g/dL are unable to donate because they do not have sufficient red blood cells to maintain oxygen-carrying capacity after the donation. Because it is difficult to find donors for patients with multiple antibodies or an antibody to a high-incidence antigen, these individuals, if anemic, may be given supplemental iron and allowed to donate once their hemoglobin levels are above 11.0 g/dL. Their cells can also be frozen for later use.

25.

C. Both Hepatitis B virus (HBV) and hepatitis C virus (HCV) are transfusion transmitted. However, only HCV is associated with hepatocellular carcinoma and cirrhosis of the liver in many of the chronically infected. Because nucleic acid testing for HCV is done on all donor samples, there is only a small risk (<1 in 2,000,000) of transmission from tested donors. The acute phase of the disease is frequently asymptomatic, but most of these patients become chronic carriers, with 70–80% having persistent infections. About 10% of those chronically infected eventually develop cirrhosis and/or hepatic carcinoma.

26.

B. Red blood cells continue to metabolize, albeit at a slower rate, during storage at 1–6°C. Decreased ATP levels result in loss of RBC viability. Plasma hemoglobin, ammonia, and K^+ levels increase, whereas plasma Na^+ and pH and 2,3-BPG levels decrease. These biochemical changes are collectively referred to as the "storage lesion" of blood.

27.

C. Because the patient received eight units of blood and none of the donors has been implicated in other cases of hepatitis, none of these donors would be deferred. The donor center should be immediately notified so it can enter in each donor's record that s/he has been implicated in a case of transfusion-transmitted hepatitis. After a second implication, the donor would be indefinitely deferred. If only one donor had been implicated, s/he would have been indefinitely deferred.

28.

C. Red blood cells and whole blood must be stored between 1 and 6°C in a monitored refrigerator with a recording thermometer and audible alarm system. During transportation between collection and transfusion facilities, blood must be packed in well-insulated containers designed to maintain a temperature range of 1–10°C. Wet ice in a leak-proof plastic bag is placed on top of the blood. The amount of ice to be used is dictated by the transportation time, the number of units packed, and the ambient outside temperature.

29.

C. In addition to the minimum number of platelets that should be present, 5.5×10^{10}, the pH of the unit must be 6.2 or higher in at least 75% of the units. The units should be assayed at the end of the allowable storage period. A donor who has taken aspirin should not be the sole donor of platelets for a patient. Aspirin has an adverse effect on platelet aggregation.

30.

C. Platelets must be stored in sufficient plasma volume to prevent the pH from falling below 6.2 at the time of expiration. Lactic acid is a by-product of anaerobic glycolysis during platelet storage, causing a drop in plasma pH and a loss of discoid shape, and hence viability. Second generation platelet bags allow better gas exchange, permitting platelets to be stored for longer periods of time at a favorable pH.

31.

C. Red blood cells expire 24 hours from the time the hermetic seal is broken, provided they are maintained at 1–6°C during the storage period. The new expiration date and time must be placed on the label and in the appropriate records. An open system exposes the blood to possible bacterial contamination. Blood may be frozen for up to 6 days after collection when maintained at 1–6°C in a closed system. If the seal is inadvertently broken on a rare unit during component preparation, the red cells may be salvaged by glycerolization and freezing, providing this is accomplished within the 24-hour restriction.

32.

D. The AABB *Standards* require that an authorized individual (such as a supervisor or medical director) review the standard operating procedures (SOPs), policies, and process annually and document the review. The SOPs should be reviewed and revised as needed to reflect the techniques used by the laboratory. It is prudent to conduct a review before a scheduled inspection and following publication of each new edition of AABB *Standards* to ensure conformance with new requirements.

33.

B. Previous ABO and Rh records of patients must be retained for 10 years and be immediately available for 12 months as a check to confirm the identity of the current pretransfusion sample. Records of unexpected antibodies identified in the serum of intended recipients and of serious adverse reactions to blood components must be retained indefinitely. Consulting records may prevent a delayed hemolytic transfusion reaction when the antibody is no longer demonstrable.

34.

D. In the United States, the weak D test is performed routinely when a donor appears to be Rh-negative, and all weak D donor units are labeled Rh-positive. Weak D units are much less immunogenic than normal D units. In many countries, neither donors nor recipients are tested for weak D.

35.

B. In order to meet the current AABB *Standards* for leukocyte reduction to prevent HLA alloimmunization or CMV transmission, the donor unit must retain at least 80% of the original red cells and the leukocytes must be reduced to less than 5×10^6. Leukocyte reduction may also prevent febrile reactions in two ways: (1) By reducing the number of leukocytes in the component to a low enough level, one can prevent febrile reactions when patients have leukocyte antibodies. (2) Cytokines are also known to cause febrile reactions. If prestorage leukocyte reduction is done, cytokine generation should be prevented.

36.

D. ABO grouping must be determined by doing both cell and serum grouping. The Rh type must be determined by direct agglutination with anti-D; if negative, the test is incubated and converted to the antiglobulin test to detect weak D phenotypes. Performing an antibody screening test on the serum or plasma of a donor is required when the donor has a history of transfusion or pregnancy. For practical purposes, most donor centers screen all donors for clinically significant antibodies. The absence of hepatitis B surface antigen (HB_sAg) and HIV must be confirmed using a method currently licensed by the FDA. The test for hepatitis B surface antibody (HB_sAb) is not required.

37.

C. In the average-size adult (70 kg), a unit of platelet concentrate should raise the platelet count by 5000–10,000/μL if there are no other complicating factors to cause decreased survival. Complicating factors include fever, sepsis, disseminated intravascular coagulation (DIC), and HLA sensitization. One apheresis platelet unit is equivalent to 6–8 units of pooled platelet concentrate and has the advantage of decreased donor exposure.

38.

A. The ABO group on all units and the Rh type on all D-negative units must be repeated by the transfusing facility for units of RBCs or whole blood collected and processed at another facility. This is generally accomplished by repeating the cell grouping only. To save time and reagent cost, it is convenient to test units labeled group O with anti-A,B only. Confirmatory testing for weak D is not required. The Rh type of units labeled D-positive need not be confirmed. Repeat antibody screening and viral testing are not required.

39–43.

(39:A, 40:D, 41:C, 42:C, 43:B) The storage temperature for whole blood, modified whole blood, RBCs, including leukocyte-reduced and deglycerolized products, is between 1 and 6°C. This range may be extended to 10°C during brief periods of transport. RBCs are frozen in a glycerol solution. These units must be stored at –65°C or lower. Fresh-frozen plasma (FFP) and cryoprecipitate are stored at –18°C or colder with a 1-year expiration. Although this temperature meets AABB *Standards,* optimal storage temperature is –30°C or below. In fact, FFP expiration may be extended to 7 years if kept at –65°C or lower. Frozen storage at low temperatures maintains optimum levels of the labile coagulation factors V and VIII in FFP and VIII in cryoprecipitate. Plasma should be frozen within 8 hours of collection when collected in CPD or CPDA-1. Platelet concentrates are stored at room temperature (20–24°C). They need to be agitated during storage.

44–48.

(44:C, 45:D, 46:D, 47:A, 48:B) Whole blood and RBCs may be stored up to 35 days when collected in CPDA-1, as long as the hermetic seal remains unbroken. Adenine added to the anticoagulant increases the viability of the cells. Cells stored only in CPD have a shorter allowable storage of 21 days. Addition of adsol solution extends expiration of red blood cells to 42 days. Fresh-frozen plasma (FFP) and cryoprecipitate expire 12 months from the date of collection if stored at –18°C or colder. The expiration time for these components is based on the deterioration of the labile factor VIII. Units stored beyond 12 months may have reduced levels of factor VIII unless stored at much lower temperatures. FFP has been approved for 7-year storage if kept at –65°C or lower. Once thawed, FFP expires in 24 hours when stored at 1–6°C. The type of plastic used in the manufacture of the bag affects the allowable storage time for platelets. The older type of bag (polyvinylchloride) does not allow as effective a gas exchange as the newer types of plastic. Platelet concentrates, prepared in PL–732 bags and stored at 20–24°C with agitation, expire 5 days from the date and time of collection.

49–52.

(49:A, 50:B, 51:C, 52:D) Blood cells continue to metabolize *in vitro.* Plasma glucose and ATP are depleted. Intermediary metabolites are generated. These may interfere with the production of energy via glycolysis. This results in a gradual loss of red blood cell viability. Storage at lowered temperatures (1–6°C) slows metabolism. ACD and CPD solutions contain sufficient glucose to support RBC viability for 21 days. CPDA-1 also contains adenine, which allows extension of the shelf life to 35 days. Adenine maintains viability by ATP regeneration. Red blood cells prepared with additive solutions such as AS-1 have a shelf life of 42 days. EDTA is not an approved solution for the storage of blood for transfusion.

53–59.

(53:D, 54:A, 55:C, 56:D, 57:D, 58:D, 59:D) Donors may be accepted after age 17, provided all results of the physical examination are normal. There is no upper age limit. Elderly donors may participate in a blood program at the discretion of the local blood bank physician. Many senior citizens obtain written permission from their personal physicians and present approval at the time of donation. The interval between donation of blood for allogeneic transfusion is 8 weeks, or 56 days. This time period is designed to protect the health of the donor. Exceptions at the discretion of the blood bank and personal physician may be made if the blood is intended for autologous use. A man who had a history of sex with another man after 1977 must be indefinitely deferred because of the possibility of transmitting the HIV virus. A history of jaundice in the first days of life is indicative of hemolytic disease of the newborn and is not a cause for deferral. A mild skin rash caused by acne, poison ivy, psoriasis, or other allergies is not a cause for donor deferral, as long as the disorder does not extend into the antecubital area at the venipuncture site. Final acceptance or deferral may be made at the phlebotomist's discretion, dependent upon whether the arm can be properly prepared to maintain sterility of the product without undue discomfort to the donor. A woman who has been pregnant is deferred until 6 weeks following conclusion of the pregnancy unless her blood is needed for her infant and the donation is physician approved.

The acceptable limits of the physical examination include:

Temperature: 37.5°C (99.5°F) or less
Pulse: 50–100 bpm
Blood pressure: systolic ≤180 mm Hg, diastolic ≤100 mm Hg

Runners or other athletes may be accepted when the pulse rate is less than 50 bpm, as long as no irregularity in beats is detected. These parameters are incorporated in the AABB *Standards* for the safety of the donor and are in general use by all blood-collecting facilities. For donor suitability, the FDA and AABB require only that the hemoglobin level be no less than 12.5 g/dL (with no sex differentiation) and that the temperature and blood pressure be within normal limits as determined by a qualified physician or by persons under his or her supervision.

60–63.

(60:D, 61:C, 62:B, 63:A) Patients with warm autoimmune hemolytic anemia (AIHA) secondary to α-methyldopa respond rapidly following cessation of the drug. They can usually be managed without transfusion. The DAT (direct antiglobulin test) may not revert to negative for up to 6 months or even longer. Leukocyte-reduced blood components ($\leq 5 \times 10^6$) are indicated in order to avoid repeated febrile episodes, CMV transmission, and alloimmunization to leukocytes. Leukocytes can be removed by filtration, centrifugation, or washing. Currently, the preferred and most efficient method is filtration with commercially available adsorption filters capable of reducing leukocytes to the required level. Patients with normovolemic anemia should be transfused with RBCs, which provide the red blood cells needed to correct the anemia in the smallest volume. These patients may not be able to tolerate whole blood because of the volume increase. It is not necessary to use leukocyte-reduced RBC for patients with normovolemic anemia. Thrombocytopenia means there is a lack of platelets. Often platelet counts drop in acute leukemia and during the subsequent treatment. Platelet counts below 20,000/μL are not uncommon under the circumstances, and the patient is considered to have severe thrombocytopenia. Leukocyte-reduced platelets will lower the chance of alloimmunization and are routinely given prophylactically to leukemia patients.

Blood Groups, Genetics, Serology

64.

D. The body makes five different immunoglobulins: IgA, IgD, IgE, IgG, and IgM. IgG makes up about 80% of the total serum immunoglobulin. Although IgA is more abundant than IgM (13% versus 6%), IgM is more common as a blood group antibody.

65.

B. From his phenotype, the father appears to be homozygous *kk* genetically. However, he is actually K^0k and has passed the K^0 gene to child 1. The K^0 gene at the *Kell* locus does not appear to result in the formation of any Kell system antigens. Child 1 has the genotype K^0K, having received the *K* gene from the mother, and has a phenotype expressing on the K antigen. Child 2 can have either the genotype *kk* or K^0k, with the mother contributing a *k* gene and the father either K^0 or *k*. The McLeod phenotype would result in weakened expression of K or k antigens. There has been no *cis-Kk* gene discovered, nor any dominant inhibitor gene that represses the expression of Kell system genes.

66.

D. All red blood cells contain some amount of H substance. The only exception is the very rare O_h (Bombay) individual because these persons lack the *H* gene that codes for H substance. Group O cells contain the most H substance, and A_1B cells contain the least amount of H substance. The order of decreasing reactivity with anti-H is: O > A_2 > A_2B > B > A_1 > A_1B.

67.

B. Two genes control Rh antigen activity. *RHD* controls the expression of D antigen, and *RHCE* determines the C, E, c, and e antigens. *RHD* is absent or inactive in D-negative individuals. Alleles of *RHCE* are *RHCe, RHcE,* and *RHce*. The RH is often dropped (for example *CE, Ce, cE, ce*).

68.

C. This individual does not have a cold autoantibody, as demonstrated by the negative autocontrol at all phases of testing. Being a nonsecretor does not affect the ABO or Rh typing, nor will it cause the appearance of unexpected antibodies in the patient's plasma. Rouleaux is ruled out because reactions are still seen at AHG after all of the patient serum or plasma has been washed away. Of the choices given, the most likely is that the patient is a Bombay phenotype individual, having inherited one *h* gene from each parent. The only other possibility is that the patient is a group O with a strong unexpected antibody or antibodies in his/her serum; however, that was not one of the choices given as an answer.

69.

B. Bombay (O_h) individuals' red blood cells not only lack A and B substances, but they also lack H substance. Bombays are genetically *hh* and, therefore, are unable to produce the precursor H substance upon which the A and B transferases act to produce A and B substances. In their serum, they will have anti-A, anti-B, anti-A,B, and an equally strong anti-H, which will react with normal group O cells. Neither O nor O_h red blood cells react with anti-A,B or anti-A_1 lectin. However, O_h red blood cells give a negative reaction with anti-H lectin, whereas O cells are positive, allowing differentiation of the two.

70.

C. The *Le* gene codes for a transferase enzyme, L-fucosyl transferase, which attaches fucose to the subterminal sugar on the Type 1 precursor substance producing Le^a substance. This occurs independently of the ABH secretor status. For Le^b as well as ABH substances to be present in the secretions, both the *Se* gene and the *Le* gene must be present. The *Se* gene produces a transferase that attaches a fucose to the terminal sugar on precursor substance, forming H substance in the secretions. Type 1H and Type 2H are the precursors for A and B substance. The *Le* gene can act upon Type 1H as well to form Le^b substance; therefore, a nonsecretor who has a *Le* gene will only secrete Le^a, whereas a secretor will secrete a little Le^a and a lot of Le^b substance.

71.

D. Monoclonal reagents containing the ES-4 clone react well with acquired B cells, and those lacking that clone do not react. Most human anti-B will react, but not the individual's own anti-B. Acquired B antigens are often associated with carcinoma of the colon, gram-negative infection, and intestinal obstructions. Also, B substance will not be found in the saliva of a person with an acquired B antigen if the patient is an ABH secretor. Acquired B occurs in group A people when microbial enzymes deacetylate the A determinant sugar (*N*-acetylgalactosamine) so that it resembles the B sugar (D-galactose).

72.

B. Lectins are proteins present in plants, often derived from the seeds of plants. Lectins can also be found in lower forms of animal life. The specificity of lectins is for carbohydrate moieties and, used undiluted, they will often react with all human red blood cells. The lectins used in blood banking are most often derived from the seeds of a plant, then diluted to achieve the desired specificity.

73.

D. The sugar L-fucose is attached to the terminal sugar of precursor substance by a fucosyl transferase. The fucosyl transferase coded by the *H* gene adds the fucose to the precursor substance on the red cells. The fucosyl transferase encoded by the *Se* gene adds a fucose to the precursor substance in the same configuration in the secretions. In both cases, the resulting configuration is called H substance. Without H substance present, the sugars giving A or B antigenic activity cannot attach.

74.

B. The complement cascade has many functions in the body associated with immunity and inflammation. The last stages of the complement cascade ultimately lead to RBC lysis. IgM is the immunoglobulin that most readily activates complement. The IgG immunoglobulins can activate complement to a lesser extent. IgG3 activates complement more efficiently than the other IgG subclasses. Although glucose-6-phosphate dehydrogenase deficiency can result in the lysis of RBCs in the presence of fava beans and certain drugs, the enzyme itself does not lyse RBCs. Albumin and antihuman globulin serum can be used in blood bank testing and do not harm RBCs.

75.

B. The answer is based upon the frequencies of genes. The genes that code for the haplotypes *DCe* and *dce* are high in the white population. A *DCe/dce* genotype has a frequency of approximately 31.1% in the general white population. The other two possible choices among the answers that would fit the typing results are *DCe/Dce* and *Dce/dCe* and have frequencies of approximately 3.4% and 0.2%, respectively. *DCe/DcE* is incorrect because the typing does not indicate that the E antigen is present.

76.

C. All these genotypes have a low frequency in the black population. *DCe/dcE* is the rarest, with a frequency of <0.1%. *DCe/DcE* is the most frequent, with an occurrence of 3.7%.

77.

A. Red blood cells that have either the C or D antigen also have the G antigen. When anti-G is made, it is capable of reacting with the G antigen on both C-positive and D-positive red blood cells, therefore appearing to be anti-C plus anti-D. In the stated case, the immunizing red blood cells were D-negative and C-positive. Therefore, what appears to be a combination of anti-D and anti-C is anti-G or a combination of anti-C and anti-G.

78.

B. The unit from the *DCe/dce* donor has the c antigen that the patient lacks. This antigen is a good immunogen. Although this patient can form the anti-E antibody, the donor cells lack the E antigen. Thus, the donor cells cannot stimulate the production of anti-E. Remember, "d" simply implies the absence of D and is not an antigen.

79.

C. Fifty percent of the children can be expected to be D-positive (*DCe/dce*) and 50% can be expected to be D-negative (*dce/dce*). The following chart clearly illustrates how the percentages were determined. The mother can pass on only *dce* haplotype, whereas the father can pass on *DCe* or *dce*.

	Father	
Mother	***DCe***	***dce***
dce	*DCe/dce*	*dce/dce*
dce	*DCe/dce*	*dce/dce*

80.

C. The husband's genotype is most likely *Dce/dCe*. He is weak D because of position effect and has a normal *D* gene. The *C* in trans position to a normal *D* gene often causes a weakened expression of D antigen. The infant has inherited the father's normal *D* gene but does not have *C* in trans position, and therefore has a normal D antigen expression. Thus, the husband is not excluded and is probably the father.

81.

C. The wife is dce/dce and, lacking the D antigen, can make anti-D. The child is D+ and therefore cannot make anti-D. The husband, although being weak D, has passed a normal D antigen to the child. This indicates that the husband's "weak D" antigen is not the result of a partial D, and therefore he cannot make anti-D.

82.

A. Occasionally, D-positive people make an apparent anti-D. The D antigen is made up of several epitopes or antigenic determinants. Those individuals missing one or more of these epitopes are called partial D. When individuals lack one or more of these epitopes, they can make an antibody, after appropriate stimulation, against the epitope or epitopes that they lack. All of these antibodies to D epitopes will react with "normal" D-positive cells that have all of the epitopes on the D antigen. Therefore, a D-positive or weak-D person appears to make an anti-D.

83.

A. The k antigen is a high-frequency or "public" antigen present in greater than 99% of the random population. The probability of encountering an individual who is k-negative and capable of producing the corresponding antibody after red blood cell stimulation is very low. Kell system antigens are good immunogens, second only to those of the Rh system. Although it is true that $Kell_{null}$ individuals are very rare, they do not make a separable anti-k.

84.

C. Anti-Jk^a often declines in the serum to below detectable levels. Therefore, when a patient has been transfused and makes anti-Jk^a and then is not transfused again for a long time, the subsequent antibody screen may not reveal the presence of the anti-Jk^a. An intravascular delayed transfusion reaction is characteristic of the Kidd antibodies because, after a second stimulation, there is a slow rise in the antibody titer and they activate complement very well. In the case of the patient in question, the antibody has been missed and he received Jk(a+) cells at some point during his stay in the other hospital. This caused a severe delayed hemolytic transfusion reaction with intravascular hemolysis.

85.

A. The Xg^a antigen is produced by a gene on the X chromosome. Because women inherit two X chromosomes, there is a higher incidence of the antigen in females. The antibody is usually detected by an antiglobulin test, and the antigenic activity is depressed by enzymes.

86.

D. To ensure that an antiserum is reacting properly, positive and negative controls must be tested. The antiserum must be tested against a cell that is negative for the corresponding antigen to ensure that no interfering substances are present that will cause false positives. It must also be tested against a cell positive for the corresponding antigen. A heterozygous cell is used to determine whether or not the antiserum will be reactive with the smaller number of antigen sites on the RBCs seen in heterozygotes. For example, when using anti-K, you would test a *kk* (K–k+) cell and a *Kk* (K+k+) cell.

87.

D. Kidd antibodies are often weak and deteriorate during storage. They are usually IgG, antiglobulin reactive only, and complement dependent. Reactions are enhanced when enzyme-treated panel cells are used. Kidd antibodies also show dosage, and the titer may drop to undetectable levels after the primary response. For this reason, they are often implicated in delayed hemolytic transfusion reactions when there is no previous record of the presence of the antibody. A Kidd antibody rarely occurs singly in a patient's serum but is often seen accompanying other antibodies.

88.

B. Enzymes denature the Fy^a and Fy^b antigens and render panel cells Fy(a–b–). Therefore, anti-Fy^a and anti-Fy^b will not react with enzyme-treated red cells. These Duffy antibodies are clinically significant. They can cause hemolytic transfusion reactions and mild hemolytic disease of the newborn. They are usually IgG antibodies and are best detected by the antiglobulin technique.

89.

B. Chido (Ch^a) and Rodgers (Rg^a) antigens are actually "pieces" of complement component 4 (C4) and are present on some RBCs, although not an RBC antigen. Anti-Ch^a and anti-Rg^a have a relatively high titer with a low avidity (HTLA) for the corresponding antigen, often not stronger than 1+ agglutination. Clinically, these antibodies are usually not significant. They are IgG and are detected by the indirect antiglobulin test. They can be neutralized with pooled normal human plasma, because complement components are always present in some amount in human plasma. Pooled plasma is used because the amount of C4 in the plasma varies between individuals. All of the other antibodies mentioned in the answers are RBC antigens and cannot be neutralized in this way.

90.

D. Anti-U is a clinically significant IgG antibody causing hemolytic transfusion reactions and hemolytic disease of the newborn. All white people appear to be U+, because no U negatives have been found. However, about 99% of blacks are U+ and 1% are U–. Those people who are U– are also S–s– and lack the entire Ss sialoglycoprotein (glycophorin B) except for very rare genetic mutations.

91.

B. Although the patient's antibody screening is negative at this time, previous records show that the patient had an anti-E. Anti-E is a significant IgG antibody; only blood negative for the E antigen should be transfused to the patient. Failure to give E-negative blood could result in a serious delayed transfusion reaction due to an anamnestic response.

92.

B. For control purposes the cell should have the weakest expression of the antigen in question; that would be an Fy(a+b+) cell. A weaker cell from a heterozygote is used because a weak antiserum might detect an antigen from a homozygote but not from a heterozygote (dosage effect). If this should happen, then red blood cells might be mistyped as Fy(a–) when in fact the cells are Fy(a+).

93.

B. Most antibodies in the Kell system are red blood cell stimulated. They are generally IgG antibodies and usually detected in the antiglobulin phase of testing. Because of their nature, they have been implicated in both transfusion reactions and hemolytic disease of the newborn. The other choices are usually IgM antibodies that cannot cross the placenta and are rarely involved in transfusion reactions.

94.

A. Antibodies can be ruled out using only one cell that is homozygously antigen positive and nonreactive at all phases of testing with the patient serum. Preferably, two or three cells that are positive for the antigen and nonreactive with the patient serum increase confidence. Antibodies in the Duffy, Kidd, and MNSs systems often show dosage. Because there are more antigenic sites on homozygous cells than on heterozygous cells, these antibodies react more strongly with homozygous cells [cells with 2 "doses" of the antigen, such as Jk(a+b−)] than with cells that carry a single "dose" of the antigen [such as Jk(a+b+)]. These antibodies can be ruled out only using homozygous cells that are positive for the antigen and do not react with the patient serum. Of the answers available, **"A"** is the best choice. Additional antibodies may not be ruled out when a standard of three cells that are positive for the antigen and nonreactive with the patient serum is used.

95.

B. The most likely combination of antibodies is anti-E plus anti-K. More than one antibody is likely because the reactions seen are of varying strengths. The panel cells that did not react with the patient serum are all lacking both the E and K antigens. The E antigen is present on Cells #4, #5, and #9. The K antigen is present on Cells #4 and #7. Note that Cell #4 has both the E and K antigens and reacts 4+ at AHG. Cells #5 and #9 have the E antigen, but not the K antigen, and both cells react 3+ at AHG. Cell #7 has the K antigen but not the E antigen and reacts 2+ at AHG. Therefore, the anti-E is stronger than the anti-K, and when both antigens are present the reaction is stronger than a reaction with one antibody alone.

96.

C. Cells #1, #4, and #7 are the cells from this panel that will be helpful in confirming the antibodies, anti-E and anti-K, and ruling out the other possible antibodies. Cell #1 is both E- and K-negative and should not react with the patient's serum. However, Cell #1 is also S+s–, Le(a+), Jk(a–b+), and Fy(a–b+) and can help to rule out all of the other possible antibodies. Cell #4 is E+, K–, S–, s+, Le(a–), Jk(b–), and Fy(b–). This cell can help confirm the presence of anti-E. Cell #7 is E–, S–, Le(a–), K+, Jk(b–), and Fy(b–). This cell can help confirm the presence of anti-K.

97.

C. Anti-Le^a, -Le^b, and -P_1 may all be neutralized by commercially available soluble substances. Le^a and Le^b are not RBC antigens but are plasma substances that are absorbed onto RBCs in the circulation. Soluble antigens are more available to the antibodies and can attach to soluble antibodies more readily than particulate antigens. Thus, the plasma (soluble) Le^a and Le^b can be used to bind to the soluble antibodies, leaving no antibody to react with the particulate antigen on the RBCs. Soluble P_1 can be obtained from several sources and can be used in the same way to preferentially bind the anti-P_1, leaving no anti-P_1 to react with the particulate P_1 antigen on the RBCs.

98.

C. Anti-N is the only antibody listed that is generally a room temperature saline agglutinin. The remaining choices, anti-Fy^a, anti-Jk^b, and anti-U, are best detected at the antiglobulin phase of testing. Remember, this is where these antibodies are optimally reactive; it does not mean they will never react at other phases of testing. Some antibodies just don't read the books!

99.

D. The Fy^a antigen is destroyed by enzyme treatment. Therefore, the anti-Fy^a seen in the initial panel will not react in an enzyme-treated panel. Enzyme-treated cells react extremely well with antibodies of the Rh system. Because the second antibody is suspected of being a weak anti-C, the antibody will react more strongly with an enzyme-treated panel than it did in the initial LISS panel. Although one cannot rule out antibodies to Duffy system, MNSs system, or Xg^a using an enzyme-treated panel, all other major blood group system antibodies present in the patient's serum should react in this medium. An elution removes antibody from the RBCs; in our scenario, the antibody is seen in the serum. Absorption of the anti-Fy^a from the patient serum could be useful; however, finding cells that lack all of the antigens lacked by the patient to avoid missing an alloantibody is a difficult and time-consuming task. Additionally, absorption will unavoidably dilute the patient's serum slightly and may dilute the weak second antibody to a point where it cannot be identified. Antigen typing the patient's cells is useful in determining whether or not the patient can form anti-C and anti-Fy^a, but it will not exclude any other antibodies nor confirm the presence of any specific antibody.

100.

B. Solid-phase red cell adherence assays, the gel test, and affinity column technology are all third-generation antibody detection methods. They have equal or greater sensitivity for clinically significant antibodies than first- and second-generation techniques. In general, they have the following advantages: less hands-on time, smaller sample size, improved safety, and stable endpoints, and they can be automated. In the gel test, the antiglobulin test does not require washing or the addition of IgG coated cells, because unbound globulins are trapped in the viscous barrier at the top of the gel column. Upon centrifugation, the anti-IgG in the column traps red cells that have been coated with IgG during the incubation period. In affinity column technology, the viscous barrier traps unbound IgG, but *Staphylococcus aureus* derived protein A and protein G are in the column instead of anti-IgG and react with the Fc portion of IgG-coated red cells. The other two techniques, solid-phase red cell adherence and polyethylene glycol, require a washing step.

101.

D. The three antibodies, anti-P_1, anti-Le^a, and anti-I, are most often non-red-cell-stimulated IgM antibodies. All of these antibodies can also, however, be stimulated by exposure to red blood cells carrying the corresponding antigen. Each of the other answers has at least one antibody that will be formed only due to exposure, either through transfusion or pregnancy, to the corresponding red cell antigen.

102.

B. Lymphocytotoxicity testing is performed by adding patient mononuclear leukocytes to wells containing sera that have antibodies to various HLA antigens and then adding guinea pig complement and indicator dye. The cells that take up the dye have had their cell membranes perforated by the action of the antigen-antibody reaction and complement, indicating that they carry the HLA antigen corresponding to the antibody in the well. Although the antibodies to Bg system antigens are antibodies to HLA antigens, the Bg terminology is only used for the remnant HLA antigens found on RBCs. The Wright system and JMH antigens are RBC antigens and are not related to the HLA system.

103.

A. Mixed-field agglutination refers to an agglutination pattern where there are two distinct cell populations, one agglutinated and one not. The appearance is clumps of cells among many unagglutinated cells. In a delayed hemolytic transfusion reaction, surviving donor cells will be coated with patient antibody and the patient's own cells will not, yielding a mixed-field DAT result. Other examples of mixed-field agglutination are seen in patients who have been transfused with blood of another ABO group, in patients with Lutheran antibodies, and in D-negative mothers with D-positive infants where there was a large fetomaternal bleed. Also, A_3 subgroup RBCs may demonstrate a mixed-field reaction with anti-A.

104.

D. The first antibody to become detectable in a primary immune response to a foreign blood group antigen is IgM followed by IgG, usually detectable from less than a week to several months after immunization. After secondary exposure to the same antigen, the antibody titer usually increases rapidly within several days. Antibodies are produced by plasma cells. The antibody produced by the plasma cells in the secondary response is IgG. Plasma cells are the terminal differentiation of the B lymphocytes. Differentiation occurs in the presence of certain cytokines when B lymphocytes are stimulated by "seeing" the antigen that corresponds to the binding site of the immunoglobulin on the B cell surface.

105.

D. All the conditions listed affect the agglutination of A and B cells in serum grouping. The gamma-globulin fraction of the serum contains the immunoglobulins. When it is reduced, there will be fewer molecules of blood group antibodies, leading to weakened or negative reactions. Both cold autoagglutinins and cold reactive IgM alloantibodies, which will react at room temperature (such as anti-M), may agglutinate the cells used because of the presence of the corresponding antigen on the group A and/or B red blood cells. Cold auto- and alloantibodies are the most common causes of ABO discrepancies.

106.

C. Blood for intrauterine transfusion should be group O, D-negative (because the fetus's blood group is unknown) and negative for the antigen corresponding to any other IgG antibody in the maternal serum. It should be recently drawn and administered as RBC (Hct 75–85%) to minimize the chance of volume overload. It should be irradiated, CMV safe, and known to lack hemoglobin S.

107.

A. Anti-Jk^a is an IgG antibody and is nearly always detected in the antiglobulin phase. Rarely, it can be detected at the 37°C phase of testing. Anti-M, anti-P_1, and anti-I are generally IgM antibodies and react at room temperature and below by direct agglutination.

108.

D. Lewis system antibodies are generally IgM. Antibodies in the Rh, Duffy, and Kell systems are generally IgG. There may be rare IgM exceptions.

109.

B. Bg antibodies react with the red blood cell equivalents of HLA antigens. Bg^a corresponds with HLA-B7, Bg^b with HLA-B17, and Bg^c with HLA-A28. These antibodies can be frustrating in that few panel cells will react and the Bg type of panel cells is often not listed.

110.

C. There is no ABO exclusion. Although the alleged father and mother are group A, they could both be heterozygous (*AO*) with the baby inheriting the *O* gene from each parent. The child appears to be of the Rh genotype *dce/dce*. One of these haplotypes is inherited from the mother. It is feasible for the alleged father to be *DCe/dce*. He could then contribute the second haplotype. The baby can inherit the *A2B12* haplotype from the mother. Although *A3* can come from the alleged father, *B15* cannot and therefore there is a direct HLA exclusion.

111–118.

(111:C, 112:A, 113:D, 114:B, 115:C, 116:D, 117:A, 118:A)

Discrepancies in ABO blood grouping may occur for numerous reasons. Any discrepancy between cell and serum grouping must be resolved before blood is identified as belonging to a particular ABO group. The presence of an acquired B antigen on cells that are normally group A can be found in some disorders, where gram-negative bacteria have entered the circulation. The serum will contain an anti-B, which will not agglutinate the patient's own cells that have the acquired B antigen. The red cell reaction with anti-B reagent may be weaker than usual. Protein abnormalities of the serum such as are present in multiple myeloma may cause the presence of what appear to be additional antibodies. The rouleaux of the red blood cells caused by the excess globulin may appear to be agglutination. Saline replacement of the serum and resuspension of the cells will usually resolve the problem in the serum grouping. Washed red blood cells should be used for the cell grouping. Infants do not begin making antibodies until they are 3 to 6 months of age. Newborns therefore will not demonstrate the expected antibody(ies) on reverse grouping. The antibody that is present is probably IgG from the mother that has crossed the placenta. An A_2 individual has the ability to make an antibody that agglutinates A_1 red cells. This anti-A_1 will cause a serum grouping discrepancy, but the antibody is almost always naturally occurring and clinically insignificant. A patient's serum may have antibodies to the yellow dye used to color anti-B reagents. If serum or plasma suspended red cells are used in the cell grouping, a false positive reaction may occur. Using washed cells will eliminate the problem. Patients who are immunodeficient may have such depressed immunoglobulins that their serum does not react with the expected A and B reagent red cells. An unexpected IgM antibody in the serum will react at room temperature and may interfere with ABO typing. Reverse grouping cells carry all of the normal RBC antigens. Therefore, they can react at room temperature with anti-M, anti-N, anti-P_1, etc. O cells may also react if they carry the antigen corresponding to the antibody in the patient's serum. Thus a patient with anti-M in his serum could react with both reverse grouping cells and the O cells if all were positive for the M antigen. A patient with cold hemagglutinin disease (CHD) may have a discrepancy affecting both cell and serum groupings. The red blood cells should be washed with warm saline before typing; the serum and reagent A and B cells should be prewarmed before mixing and testing and converted to the antiglobulin test if necessary.

119–123.

(119:C, 120:A, 121:A, 122:D, 123:B) The Kell system has a number of antigens, among which is Kp^a (Penney). This antigen has not been reported in blacks. The corresponding antibody is very rare because so few individuals have the antigen that stimulates its production. When it is present, it is not a serious problem because Kp(a–) blood is easily found. The McLeod phenotype is one in which all the Kell-associated antigens are expressed only weakly. McLeod cells are missing a precursor substance called Kx. Kx is coded for by a gene present on the X chromosome. Some of the male children afflicted with chronic granulomatous disease are of the McLeod phenotype, but exactly how the two are associated is not clear. The *Ss* locus is closely linked with the *MN* locus, and they are considered part of the same blood group system. M^g (Gilfeather) is a rare allele in the MN system. When the M^g antigen is present it can cause typing difficulties because it will not react with either anti-M or anti-N. Because the MN antigens are well developed at birth, they were often used in paternity testing. The presence of an M^gM or M^gN combination can look like a homozygous *M* or *N*, leading to a second-order (indirect) exclusion unless the red cells are tested with anti-M^g. The presence of the M^g antigen on the red blood cells of the alleged father and child practically proves paternity. Currently, most paternity testing is done by DNA analysis, not by red cell antigen testing.

Antibody Identification, Transfusion Therapy, Transfusion Reactions

124.

A. The racial origin of this donor is probably black. This origin can be determined by looking at the Duffy (Fy) phenotype. About 70% of American blacks are Fy(a–b–). This phenotype is extremely rare in whites.

125.

D. Donor 5 is homozygous for the following genes: *Ce, s, k,* Jk^a, Fy^a, because the corresponding antigen is produced and the antithetical antigen is not being produced by an allele (for example, C+c– implies homozygosity: *CC*). The donor cannot be homozygous for *M*, because its allele is producing N antigen. There is no way to tell whether P_1 is homozygous, because it lacks a co-dominant allele and P_1 does not show dosage. There is no Le^b gene. The antigen is produced by the action of the *Le* gene on Type 1 H. The Lewis genes are *Le* and the amorph *le*, and dosage is not observed.

126.

A. Anti-Fy^a can be identified by eliminating specificities where the corresponding antigens appear on the panel cells that do not react. The differences in the strength of reactivity can be explained by the fact that the Duffy antigens show dosage (react stronger with cells from homozygotes). Cells #1 and #6 are from Fy^a heterozygotes [Fy(a+b+)]. Cells #4 and #5 are from Fy^a homozygotes [Fy(a+b−)]. When eliminating an antibody specificity known to show dosage, it is best to have a negative reaction with a panel cell from a donor who is homozygous for the corresponding gene. Fy^a and Fy^b antigens are destroyed by enzymes. Although the Fy(a–b–) type is common in blacks, the frequency of Fy^a in whites is about 66%. Anti-E and anti-s should be ruled out with Fy(a–) cells from individuals who are homozygous for *E* and *s* (in other words, E+e– and S–s+).

127.

C. Serum must be present to cause rouleaux formation; it should not occur at the antiglobulin phase of testing when the rouleaux-producing properties have been removed by washing. Warm and cold autoantibodies result in a positive autocontrol, usually equal in strength to reactivity observed with reagent red cells. Antibodies directed against preservatives in potentiating media should also react in the autocontrol. When the autocontrol is nonreactive and all panel cells are uniformly positive, one should suspect the presence of an alloantibody directed against a "public," or high-frequency, antigen. A selected panel of red cells, each lacking a different high-frequency antigen, should be tested until a compatible cell is found. The patient's red cells may be typed for a variety of high-frequency antigens. If such an antigen is found to be missing on the red cells, the corresponding serum antibody is likely that specificity.

128.

B. Cell #7 is negative for the high-frequency antigen k (cellano). Many other specificities cannot be ruled out because there is only one negative reaction. Treating the panel cells with dithiothreitol (DTT) destroys Kell system antigens. If no reactions are seen when the panel is repeated with DTT-treated cells, then many other clinically significant antibodies can be ruled out and the presence of anti-k would be supported. If the patient has not recently been transfused, his cells should be typed with anti-k and would be expected to be k-negative. Proteolytic enzymes neither destroy Kell system antigens nor enhance their reactions with Kell system antibodies. Treating serum with DTT will destroy IgM antibodies by cleaving disulfide bonds of the pentamer and would not be helpful because anti-k is generally IgG.

129.

C. From the presence of positive reactions taking place at two different temperatures, it appears that there are two different antibodies reacting. There is a cold antibody reacting with Cells #3 and #8 at immediate spin and a warm antibody reacting with Cells #1, #2, #3, and #4. It is unlikely that the cold antibody is carrying over to a warmer phase, because there is no 37°C reaction with Cell #8.

130.

C. Anti-Le^a, -Le^b, and -P_1 are antibodies that react at immediate spin (room temperature or below). Of these, P_1 and Le^b antigens are present on Cell #7, which shows negative reactivity. This makes these specificities unlikely to be present in the patient's serum. Le^a antigen is present on Cells #3 and #8, both of which show a positive immediate-spin reaction. Anti-D is usually IgG and reacts best at 37°C and AHG phases of testing.

131.

B. All the antibodies listed react at warm temperatures. The K antigen is present only on Cells #1 and #7 and is absent from Cells #2, #3, and #4 that reacted at 37°C and AHG phases of testing. Also, Anti-K and anti-k do not usually react without the addition of AHG. Anti-C and -D may react at 37°C without AHG, but usually only if albumin or enzymes are used as potentiators. Anti-C and -D are often found together. In this instance, however, there would be a positive reaction with Cell #5 if anti-C were present as well as anti-D.

132.

D. A patient's red blood cells should be negative for the antigen corresponding to the antibody identified as long as the autocontrol is also negative. In this case, one already knows that the patient is group A, D-negative (does not have D antigen). A standard approach has been to require three antigen-positive cells that react and three antigen-negative cells that do not react for each antibody identified to establish probability that the antibody(ies) has (have) been correctly identified. There are only two Le(a+) donor cells on this panel. The anti-Le^a reacts only at immediate spin and the anti-D does not. Presumably the screening cells have an additional Le(a+) cell. Because this antibody appears to be clinically insignificant, many would simply ignore it by eliminating the IS. At any rate, it would certainly not be necessary to run another panel.

133.

B. The patient's positive antibody screening test is consistent with an anti-K, and this is what was identified in the antibody identification. Three K antigen-positive and three K antigen-negative cells were tested and reacted appropriately. The antibody identification could have been misinterpreted, but it seems unlikely. The panel must have been read at the AHG phase of testing, because most examples of anti-K do not react at any other phase of testing. Positive and negative control cells (K+k+ and K–k+) should be tested with the anti-K at the same time as the patient's cells to be certain of the specificity of the anti-K antiserum. There is no indication that this has been done, and the patient's phenotype should not be K+. If the patient had circulating K+ donor cells, the K typing would have shown a mixed-field reaction, which has not been indicated.

134.

B. Although there are many potential sources for error in performing an indirect antiglobulin test, the most common error leading to a false negative reaction is the failure to wash the red blood cells adequately before the addition of AHG reagent. Traces of free human globulin can neutralize the AHG reagent. Red cells known to be coated with IgG antibody (Coombs' control cells, check cells) are added to all negative tests. Agglutination of these control cells confirms that AHG was present in the system and that proper washing procedures were performed.

135.

C. Elution is a process in which bound antibody is released from red blood cells. The eluate produced can then be further tested to identify the specificity of the antibody. Some elution methods use temperature, chemicals, or manipulation of the pH to dissociate antibodies from red cells.

136.

C. If the antiglobulin test was performed properly and the antiglobulin reagent is working properly, the IgG-coated control red blood cells should be agglutinated; thus, this test is invalid. Unagglutinated cells after the addition of the control cells might mean that the cells were not washed well and that the antiglobulin reagent has been neutralized or that the antiglobulin reagent may have been omitted. The test must be repeated if this happens.

137.

B. The crossmatch is performed by testing the serum of the recipient with a suspension of the donor's red blood cells. The serum and red cells are usually tested at the immediate-spin (IS) phase to detect ABO mismatches, if the patient has no history of having unexpected antibodies in his/her serum and the current antibody screen is negative. Additional testing is done at 37°C and antiglobulin phases to detect the presence of clinically significant antibodies, if the patient has a positive antibody screen or a history of ever having an unexpected antibody in his/her serum. Because clinically significant antibodies (other than anti-A and anti-B) are almost always detected during the antibody screening test, AABB *Standards* sanctions performing only the immediate-spin crossmatch (for ABO compatibility) when the patient has a negative antibody screening test. An antiglobulin crossmatch *must be performed* when a patient has a positive antibody screening test because of a clinically significant antibody, or if the patient has a history of a clinically significant antibody. Compatible units must also be phenotyped for the corresponding antigen and shown to be negative. When an antiglobulin crossmatch is performed, potentiating media such as albumin, polyethylene glycol (PEG), or LISS may be added to the test system to enhance sensitivity and/or decrease incubation time.

138.

B. A group AB individual can receive red blood cells from donors of all ABO groups. Because the patient does not have anti-D, it would be best to next select group A, D-positive units because the need for large amounts of blood is anticipated. These units should be given as RBCs, because the plasma has anti-B. If necessary, the patient may be later switched to group O, D-positive RBCs. It would not be wise to deplete the D-negative supply, because D-negative women of child-bearing age may need blood and should not be exposed to the D antigen. The decision to transfuse D-positive blood to a D-negative patient must be approved by the physician in charge of the transfusion service.

139.

D. The crossmatch, which is the recipient's serum with the donor's cells, will reveal only if the patient has a detectable antibody against some antigen on the donor cells. In the presence of a negative antibody screening test, an incompatible crossmatch at the immediate-spin phase will most likely be due to an ABO mismatch between the recipient's serum and the donor's cells. For this reason, AABB *Standards* mandate performing only the immediate-spin crossmatch when the patient has a negative antibody screening test and no history of clinically significant antibodies. The crossmatch will not guarantee *in vivo* response to the transfused red blood cells. Also, it will not detect all ABO typing errors, and it will not detect most Rh typing errors.

140.

C. A false positive crossmatch could occur if the donor has a positive direct antiglobulin test (DAT). A DAT should be done on the donor cells and, if positive, the unit should be removed from inventory. Another possible cause of a false positive crossmatch could be contaminants in dirty glassware causing clumping of red cells. The other responses are true positives. If a strong incompatibility is immediately present, one should check the ABO type of the patient and the donor. If the antibody screening test was negative, one might suspect an antibody against a low-incidence antigen on the donor's cells.

141.

C. The crossmatch consists of testing donor cells with recipient serum. A group A individual will have anti-B in his/her serum, which will agglutinate AB cells. D-positive cells given to a D-negative person may cause antibody stimulation, but there will not be a visible reaction without a preformed antibody.

142.

A. The most critical step to ensuring safe transfusion is obtaining a properly labeled blood sample from the correct patient. Transfusion accidents due to ABO mismatches are usually the result of a patient receiving the wrong blood. The identity of the patient must be verified, both verbally and by comparison of the wristband with the transfusion request form. Tubes must be labeled properly at the bedside with the full name, another acceptable identifier such as the medical record number, and the date.

143.

B. Sufficient information for unique identification of the patient (including two independent identifiers) and the date of sample collection must be on the label. The phlebotomist's signature or initials must appear on either the tube of blood or on the request slips. It is not necessary for both to be signed. The physician's name, the patient's room number, and the time of the phlebotomy may be helpful but are not required by AABB *Standards*.

144.

A. According to AABB *Standards,* specimens used for antibody screening and crossmatching must be less than 3 days old if the patient has been transfused or pregnant within the past 3 months. Either serum or plasma may be used. The specimen must be labeled properly at the bedside at the time of collection. Specimens are required to be retained for only 7 days posttransfusion.

145.

B. In general, one unit of red blood cells should raise a patient's hemoglobin by 1 g/dL. In this instance, a 2-g/dL rise is required, so two units would need to be given. This rule is true for patients of average size. A very large or heavy individual with an expanded blood volume may require additional units to attain the same level. Conversely, a pediatric patient may require less.

146.

B. The percentage of compatible blood is obtained by multiplying the frequencies of antigen negative. In this instance, one wants to find Jk(a–), K– blood. The incidence of Jk(a+) blood is 77%; therefore, the incidence of Jk(a–) blood is 23%. Likewise, K+ incidence is 10%; K– would be 90%. Multiply these two frequencies together to get the frequency for Jk(a–), K– units: $0.23 \times 0.90 = 0.21$, or an incidence of 21 units in 100. Divide 2 by this figure because 2 units are needed: $2 \div 0.21 = 9.5$, or 10 units must be screened to find 2 compatible units.

147.

C. Cryoprecipitate provides a source of fibrinogen and fibronectin in addition to factors VIII and XIII. This component is indicated for use in bleeding disorders associated with hypofibrinogenemia, such as DIC, when excessive fibrinogen consumption is occurring. Each unit contains an average of 250 mg of fibrinogen or 0.25 g. The AABB *Standards* require a minimum of 150 mg per individual collection. The amount of pooled product to administer is calculated by the formula:

$$\frac{\text{Total grams desired}}{0.25\ \text{g/unit}} = \text{Total number of cryoprecipitate units to administer}$$

For example,

$$\frac{2\ \text{g}}{0.25\ \text{g/unit}} = 8\ \text{Units}$$

148.

D. Plasma compatible with the recipient's ABO group is preferred when large volumes are transfused. Both group O and group B plasma contain anti-A that can cause a positive direct antiglobulin test (DAT) when infused into either group A or AB recipients. Compatibility testing is not required before cryoprecipitate administration. Plasma compatibility is not as important with cryoprecipitate as with platelet concentrates. Approximately 10 mL of plasma is in a cryo unit and 50 mL in a single platelet concentrate.

149.

D. Approximately 1% of transfused red cells are cleared daily from the circulation of a recipient. The clearance rate may be increased in patients with autoimmune hemolytic anemia, pernicious anemia, aplastic anemia, hemorrhage, splenomegaly, and fever. Transfused cells survive normally in patients with anemia because of intrinsic red cell enzyme defects, spherocytosis, and paroxysmal nocturnal hemoglobinuria.

150.

B. Febrile reactions are brought about by the interaction of antibodies in the recipient directed against antigens on donor leukocytes or by cytokines secreted by leukocytes. The antigens involved are both the HLA and granulocyte-specific antigens. Leukocyte-reduced RBCs are the component of choice for a patient with repeated febrile transfusion reactions. Although frozen RBCs that have been thawed and deglycerolized are considered leukocyte reduced, the cost and time involved in preparation make them an unpractical choice.

151.

D. Red blood cells are the component of choice to maintain or restore oxygen-carrying capacity. This component has the least effect on blood volume and the maximum effect on the oxygen-carrying capacity of all the products available for transfusion. In some patients, increasing the total blood volume more than what is absolutely necessary could have a detrimental effect. Examples are patients with chronic anemia or congestive heart failure.

152.

C. Children inherit half their genetic characteristics from each parent. Because the parents are not identical in antigen composition (a situation only found in identical twins), the child cannot be totally compatible with either parent. Siblings, however, have access to the same genetic material from each parent and so may have identical genes and antigens. A spouse genetically would be equivalent to a random donor.

153.

D. Because time is of the essence when a trauma victim is severely hemorrhaging, blood bank personnel must respond promptly. Group-specific blood may not be issued on the basis of previous patient or donor records. If the situation is so urgent as to preclude performing an ABO and Rh typing, or when a blood sample cannot be obtained, group O RBCs may be issued. The decision as to whether group O, D-positive or O, D-negative RBCs should be used will depend upon inventory and the age and sex of the trauma victim. Blood banks located in a trauma center should have a written procedures manual with well-defined criteria. All staff must be familiar with these guidelines.

154.

B. The first step in any pretransfusion work-up is to check the blood bank records for previous information on the patient. This information must match the ABO group and Rh type on the sample obtained currently. This helps to ensure that patients are not given the wrong blood type. A mismatch may indicate that the wrong patient was drawn or the label was incorrectly applied. After the initial release of 6 units of uncrossmatched group O blood, there should be ample time to at least check records and obtain an ABO group and Rh type on the patient sample before more blood is required.

155.

D. Large volumes of transfused plasma should be ABO compatible with the recipient's red blood cells. Isoagglutinins present in the plasma will attach to the corresponding antigen on the patient's red cells *in vivo* and cause a positive DAT and perhaps hemolysis. Plasma of any blood group can be given to a group O patient, because his/her red cells will not be agglutinated by anti-A or anti-B in donor plasma.

156.

B. Physiologic saline is the only generally acceptable solution that is allowed to be added to blood or blood components. Ringer's solution causes small clots to develop in anticoagulated blood, and 5% dextrose causes red cell lysis. Other solutions and medication should not be added to blood unless they have been proved safe and are sanctioned by the FDA.

157.

C. The majority of deaths due to hemolytic transfusion reactions are caused by clerical errors, not laboratory errors. Patients, blood samples, and lab records, if misidentified, may lead to the wrong ABO type blood being administered to the patient. These deaths most often occur in areas of high stress, such as in emergency departments and surgical suites.

158.

C. A delayed hemolytic transfusion reaction is generally the result of a patient's second exposure to an antigen present on donor red blood cells. The patient at some time previously had been exposed to the antigen, and this is his/her anamnestic response. This reaction usually occurs from 3 to 14 days after transfusion and is accompanied by extravascular red blood cell destruction. Often the patient is asymptomatic. The DAT is usually positive in a delayed hemolytic reaction, because the reaction is extravascular and coated cells are present in the peripheral circulation. An acute hemolytic transfusion reaction is usually intravascular, and the coated cells are destroyed by complement, leaving the DAT negative or at most weakly positive. Anaphylactic and febrile reactions do not involve red blood cell antibodies and do not cause a positive DAT.

159.

C. Kidd antibodies are generally IgG, complement dependent, and warm reacting. However, they are usually weak and labile. Because of this, they may go undetected in pretransfusion testing and the patient may inadvertently be transfused with antigen-positive blood, leading to a delayed transfusion reaction.

160.

A. Some people are genetically deficient in IgA. If these individuals have anti-IgA in their plasma, they may suffer a severe anaphylactic reaction when subsequently exposed to IgA in donor plasma. Once these people are identified, they must receive IgA-deficient components such as multiple-washed or frozen-thawed RBC or components drawn from IgA-deficient donors.

161.

A. Viral inactivation methods such as the use of a solvent/detergent combination have eliminated the risk of transmission of viruses with a lipid envelope in clotting factor concentrates. This method has been applied to group-specific frozen plasma. Pooled plasma, solvent/detergent-treated is much safer than the other components listed from the standpoint of HIV, HBV, and HCV, because the process destroys lipid-enveloped viruses. It does not destroy non-lipid-enveloped viruses such as parvovirus B19. However, it has been withdrawn from the market in the United States and is not currently being used. Another approach to safety is "FFP-Donor Retested," which means that the FFP (fresh-frozen plasma) has been held for 90 days or more and released only after the donor has been retested negative for infectious disease markers. It is not a pooled product. The retesting should show that the donor was not in an infectious window period when the plasma was drawn.

162.

C. The transfusion of the unit should be stopped, and the transfusionist should keep the patient's intravenous (IV) line open with physiologic saline in case medications must be given quickly to counteract the transfusion reaction. The unit must then be returned to the blood bank along with all of the transfusion set and any attached IVs, such as any physiologic saline that was being infused along with the unit. The patient's physician should be notified, but after the transfusion is discontinued and the new IV of physiologic saline has been hung. Monitoring pulse and blood pressure is a good idea but is not an immediate necessity.

163.

D. Returning the unit to the blood bank and obtaining posttransfusion blood and urine samples is required by AABB *Standards*. No more blood or blood products should be transfused into the patient until the reaction has been investigated. If the patient has an antibody that caused the transfusion reaction, any red blood cell units transfused must be negative for the corresponding antigen.

164.

B. Red-tinged plasma is indicative of hemolysis. When this is seen in the posttransfusion sample but not in the pretransfusion sample, it is evidence that an intravascular hemolytic transfusion reaction has occurred. The antibody attached to antigen on the patient's RBCs, and the resulting antigen-antibody complex activated the complement cascade. This resulted in hemolysis of the coated RBCs in the patient's vasculature. Other likely laboratory findings in this situation include hemoglobinuria and decreased plasma haptoglobin. Although intravascular hemolysis can be seen in situations where a drug has been administered with the blood, there are several possible causes of an intravascular hemolytic transfusion reaction. In the scenario given, there is no evidence that would suggest any particular cause.

165.

B. Autologous transfusions are the safest form of transfusion available, although they are not always the most practical. When administered properly, they eliminate disease transmission, immunization to foreign antigens, allergic reactions, and graft-versus-host reactions. Clerical error is still a significant risk. An AABB survey revealed that 1.2% of respondents reported an erroneous autologous transfusion. Although preoperative autologous collection is feasible for elective surgery, this form of autologous transfusion is not possible in cases of unexpected or massive blood loss. Intraoperative blood collection is another form of autologous transfusion used during operations where the estimated blood loss is great. Another advantage of autologous transfusions is that allogeneic donor blood is available for other patients.

166.

D. Reviewing the previous records of a patient may help to confirm the identity of the current pretransfusion sample. Records should be checked for ABO group and Rh type, clinically significant antibodies that were present but may no longer be detectable, and adverse reactions to previous units transfused. ABO records from the past 12 months must be immediately available and retained for 5 years; antibody and adverse reaction records must be available indefinitely for review before issuing blood for transfusion. It is not necessary to check hepatitis records of the patient because the hepatitis status of the patient does not affect transfusion of blood to him as a recipient.

167.

C. The crossmatch is performed using the recipient's serum and the donor's red blood cells. Therefore, a positive DAT on the recipient's cells will not affect the crossmatch results. A positive reaction may be obtained when the recipient has an antibody directed against a corresponding antigen on the donor's red cells. If this is a low-frequency antigen, the crossmatch may be incompatible and the antibody screening result negative. A positive reaction may also indicate that the donor's red cells are coated with human globulin. This can be confirmed by performing a direct antiglobulin test (DAT) on the donor's red cells. Units of blood demonstrating a positive DAT should be returned to the collecting facility.

168.

D. Monoclonal anti-D reagents are low-protein reagents, therefore, a negative reaction with anti-A and/or anti-B (also low-protein) serves as a control. When the patient appears to be group AB, D-positive, it is necessary to set up a separate control. A drop of the patient's cell suspension with his/her own serum (autocontrol) or with 6–8% albumin makes a suitable control.

169.

D. Both the ABO grouping and Rh typing are in question. Because the transfusion need is urgent, group O, D-negative donor units should be selected initially for this young woman of childbearing age. They should be transfused, if necessary, before the problem has been resolved or crossmatching performed. In some cases, the risk of withholding transfusion is far greater than the risk of a transfusion reaction in a patient with an unresolved antibody problem. The physician must sign an emergency release form indicating that the clinical situation was such to warrant the release of blood.

170.

B. The patient most likely has a potent cold autoagglutinin. The antibody screening test and crossmatches with group O, D-negative donor units should be set up as soon as possible by prewarmed technique. In the past, when Rh typing was primarily done with high-protein reagents, an Rh control, containing all the potentiating ingredients found in the Rh reagent except for the anti-D, was tested in parallel. The most likely cause of a positive Rh control with a high-protein reagent is a strongly positive DAT result. This would not be the cause in this case because monoclonal anti-D is a low-protein reagent. The usual cause of false positive reactions with low-protein reagents is a potent cold autoagglutinin. A single wash may not remove all the antibody from the patient's red cells. The cells should be washed with warm saline; and if they are still autoagglutinated, antibody can be removed by 45°C heat elution or treatment with a sulfhydryl reagent such as dithiothreitol (DTT), which destroys IgM antibodies. Because washed red cells were used when typing the patient's red cells, multiple myeloma could not be the cause of the false positive, because the abnormal protein causing the pseudo-agglutination (rouleaux) would have been washed away.

171.

C. Although rigors and shock may be caused by hemolytic or anaphylactic reactions, bacterial sepsis is the most likely cause in this case. The sudden rise of the patient's temperature from normal to 40°C or above is typical of such an infection. Bacterial sepsis is an important cause of transfusion reactions, with about one-fourth of these reactions resulting in death.

172.

D. The incidence of bacterial sepsis is highest with platelet components. It is higher with pooled platelets than platelets collected by apheresis. Pooled platelets usually involve 6 or more donations from different donors, multiplying the chance of contamination. Most bacteria grow better at room temperature (the normal storage temperature for platelets) than refrigerator temperature. Sepsis from RBCs is usually due to *Yersinia enterocolitica,* which grows well at refrigerator temperature.

173.

B. The reactions are most likely all caused by the cold autoagglutinin anti-I. The I antigen is not present on *ii* cells. Autoadsorption of the patient's serum with his/her own cells should not be performed following recent transfusion. Alloantibody may be adsorbed onto circulating donor red cells, resulting in false negative reactions with repeat testing of the autoadsorbed serum and reagent red cells. The weak reactions at the AHG phase of testing are most likely due to complement being bound at room temperature by the cold autoantibody reacting with the anti-C3d in polyspecific AHG reagent. A prewarmed technique, in which the donor's cells and patient's serum are warmed separately to 37°C before combining, is commonly used to eliminate interference from cold agglutinins. Many transfusion services use an anti-IgG monospecific AHG reagent, instead of a polyspecific reagent that contains anti-IgG and anti-C3d, in order to avoid such problems, but the prewarmed crossmatch should eliminate complement from being bound. Because the patient was recently transfused, there is a slight possibility that the reactions at AHG could be caused by a high-incidence alloantibody causing delayed hemolysis. Such an antibody would still react by prewarmed technique.

174–179.

(174:A, 175:A, 176:E, 177:D, 178:D, 179:C) The ABO group and Rh type must be determined by the blood-collecting facility with every donation. The unit must be labeled using the interpretation of current testing, not with previous donor records from repeat donors. When the immediate-spin (IS) reaction of the donor red cells is positive with anti-D (with a negative Rh control), the unit may be labeled D-positive. If the red cells fail to agglutinate anti-D directly, the test must be incubated and converted to the antiglobulin test to detect weak D phenotype. All units tested with anti-D that are IS negative but are found to be weak D-positive must be labeled D-positive to avoid sensitizing an intended D-negative recipient to the D antigen. A direct antiglobulin test (DAT) should be performed as a control along with the weak D test. For the test to be valid, the DAT must be negative. If the donor is DAT-positive, the weak D status cannot be interpreted because the donor's red cells are coated with antibody before the incubation with anti-D. DAT-positive units of blood should be discarded. Two different test methods, a cell grouping and a serum grouping, must be used for ABO grouping; the results of these methods must be in agreement before a label is applied to the unit. Although testing the red cells with anti-A,B and testing the serum with A_2 red cells is not required, many collecting facilities incorporate these additional reagents to detect discrepancies due to subgroups of A or B. When the cell and serum groupings are not in agreement, additional testing to resolve the discrepancy is required. Weak or missing red-cell reactions with anti-A,B or anti-A, or both, accompanied by serum reactions with A_1 cells, but not A_2 cells, are an indication that the donor may be a subgroup of A with anti-A_1. Extended incubation of the cell grouping, testing with additional A_1 cells, A_2 cells, and anti-A_1 lectin, and adsorption/elution/titration/secretor studies are techniques used to resolve discrepancies due to subgroups. Donor units found to contain unexpected antibodies should be processed into RBCs with small amounts of plasma. They should be labeled to indicate the antibody specificity. It is helpful to attach a tie tag with this information to the RBCs. Transfusing large amounts of antibody containing plasma (such as anti-Fy^a) into a Fy(a+) recipient may cause decreased red cell survival and, therefore, is not used for individual transfusion to patients. Plasma from units with antibodies may be salvaged for reagent use or source plasma.

180–184.

(180:C, 181:C, 182:D, 183:D, 184:A) There are three parts to the factor VIII molecule: F VIII:C, F VIII:Ag, and F VIII:vW. Individuals manifesting the X-linked (gene carried on the X chromosome) disorder known as hemophilia A are deficient in F VIII:C. The clinical severity, resulting in hemorrhage either spontaneously or following trauma, depends upon the level of F VIII:C present. Deficiency in F VIII:vW is known as von Willebrand disease. It is not X-linked and is the most common inherited coagulopathy. Deficiency in F VIII:vW results in impaired platelet adhesion and aggregation, leading to prolonged bleeding. Cryoprecipitate contains both F VIII:C and F VIII:vW and may be used for treatment of these disorders, although it is not the preferred treatment. F VIII concentrates have become safer with improved viral inactivation processes, and some now have therapeutic amounts of F VIII:vW as well. Cryoprecipitate also contains an average of 250 mg of fibrinogen per unit, as well as Factor XIII and fibronectin, and currently it is primarily used to treat hypofibrinogenemia. Although Factor V deficiency is rare, it can present severe manifestations leading to hemarthrosis. Treatment of choice is fresh-frozen plasma (FFP) because F V is a labile factor not found in cryoprecipitate. FFP can be used to correct the factor deficiencies found in liver disease (factors II, VII, IX, and X). Because all these are stable factors, the plasma need not be fresh even though FFP is commonly used. Platelet concentrates are used to correct thrombocytopenia following chemotherapy. Fresh whole blood is seldom available. Specific components are instead provided to give the patient exactly what is needed and conserve blood resources.

Hemolytic Disease (Hemolytic Disease of the Newborn, Immune Hemolytic Anemia)

185.

C. ABO testing, Rh testing (for weak D when applicable), and antibody screening should all be performed early in a pregnancy. Amniocentesis should be done only when clinically indicated. Furthermore, amniocentesis generally is not done before the third trimester, although in recent years the procedure has been done as early as 14 weeks.

186.

C. The Lewis typings of a pregnant woman may appear to be Le(a–b–), even though the original typing may have been Le(a–b+). When women are pregnant, they have an increased plasma volume and increased amount of lipoprotein in relation to red blood cell mass. Because Lewis antigens are adsorbed onto red cells and lipoprotein from plasma, the dilutional effect and greater lipoprotein mass would lead to less adsorption of Le^b onto red cells. After the pregnancy, the woman will return to her original type.

187.

A. Prenatal testing for all pregnant women should include ABO, Rh, and antibody screening to exclude the presence of unexpected antibodies with the potential for causing hemolytic disease of the newborn (HDN). The presence of an unexpected antibody does not indicate that the infant will be affected. Testing the red blood cells of the father, to determine whether the corresponding antigen is expressed and, if so, whether he is a homozygote or heterozygote, should indicate the probability for the presence of the antigen on infant cells. ABO-HDN is not predictable until postpartum, when the blood type of the infant is determined.

188.

D. IgG is the only immunoglobulin that is transported across the placenta. It does not cross the placenta because of low molecular weight or simple diffusion, as evidenced by higher concentrations of antibody present in cord than in maternal serum. IgG molecules are actively transported via the Fc portion beginning in the second trimester. Therefore, potentially any IgG blood group antibody produced by the mother could cause HDN, if the fetus possesses a well-developed corresponding antigen. The disease varies widely in severity, being dependent on multiple factors.

189.

A. Although the ABO system is most often implicated in fetomaternal incompatibilities, it very rarely causes clinical symptoms. ABO-HDN generally occurs when group O mothers have group A or B children. Although Rh-HDN can be prevented, there is no prevention for ABO-HDN, and generally there is none needed because exchange transfusion is rarely necessary.

190.

C. When D+ red blood cells are sufficiently coated with antibody, leaving no or few remaining sites to react with D antiserum, the cells are referred to as having a "blocked D" and may react weakly or not at all with a low-protein anti-D reagent. One may suspect this phenomenon, confirmed by elution of anti-D from the red cells, when the DAT is strongly positive. Enough antibody may be removed either with a gentle heat elution (45°C) or using chloroquine diphosphate to permit accurate D typing of the coated red cells.

191.

C. Given that all prenatal and neonatal testing is valid, one should consider an antibody against a low-incidence antigen. The low-incidence antigen was of paternal origin, and it stimulated the mother to form an IgG antibody. To prove this theory, an eluate from the baby's cells should be tested with the father's cells. Also, the mother's serum and the baby's eluate could be tested with a panel of cells positive for various low-incidence antigens to identify the specificity of the antibody.

192.

D. An antibody panel performed on an eluate made from the baby's red blood cells is the most conclusive way to identify positively the antibody causing the positive DAT. This would be especially helpful in a case where the mother has several antibodies that could cause hemolytic disease of the newborn. However, RBCs for transfusion in the neonatal period should be negative for any antigen corresponding to any IgG antibody that crossed the placenta.

193.

B. During the first few hours of life, the primary risk to a baby with hemolytic disease of the newborn is heart failure caused by severe anemia. After the first 24 hours, in which the anemia can be compensated, the highest risk to the infant comes from hyperbilirubinemia. Kernicterus, which is brought on by hyperbilirubinemia (generally >18 mg/dL of unconjugated bilirubin) in a full-term infant in the first days of life, can cause irreversible brain damage. Depending on the severity of the hyperbilirubinemia, one or more exchange transfusions may be needed.

194.

D. A positive DAT on the infant's red blood cells indicates that IgG antibody has crossed the placenta and coated the neonate's red cells. Identification of the antibody in the maternal serum and elution of the same antibody from the infant's red cells confirms the specificity of the offending antibody. In lieu of a maternal blood sample, the identity of the antibody may be confirmed by testing the infant's eluate and serum. The eluate contains the antibody(ies) responsible for the clinical HDN; the serum may contain additional maternal antibody(ies) directed against antigens absent on the infant's cells but present on the donor's cells. Blood compatible with both serum and eluate should be prepared for exchange transfusion.

195.

D. Providing blood of the baby's type is exactly what one does not want to do. This would defeat the purpose of the exchange transfusion. For example, if a D+ infant was suffering from HDN because of an anti-D, the transfusion of D-positive cells would allow transfused cells to be coated with anti-D. The transfused cells would then be removed from the circulation by the RES and would have decreased survival.

196.

D. A mixed-field weak D test on maternal blood indicates the presence of D-positive baby cells circulating with the mother's D-negative cells, suggestive of a large fetomaternal hemorrhage. If a mother does demonstrate a positive weak D test when previously it was negative, a Kleihauer-Betke acid elution test should be done on the mother's red blood cells. This test is used to quantify the amount of the fetal blood that has entered the mother's circulation. The results of the test will determine how many vials of Rh immune globulin should be administered to the patient. One vial will protect against approximately 30 mL of fetal blood (or 15 mL of red cells) that have entered the mother's circulation. A more sensitive method to identify fetomaternal hemorrhage (FMH) than the test for weak D is the rosette test. A maternal red cell suspension is incubated with an anti-D of human source, allowing antibody to coat D+ fetal cells. D+ indicator cells are added that bind to the coated D+ cells, forming rosettes. This is a qualitative test and must also be followed by a quantitative test, such as the Kleihauer-Betke acid elution test.

197.

C. The most likely cause for the positive antibody screening test is the presence of a passively acquired anti-D. Because the mother received antepartum Rh immune globulin (RhIG), anti-D from that injection may still be present at childbirth. Depending on how the RhIG was injected postpartum, the anti-D could already be present in the patient's serum. Because antepartum RhIG was given, it is unlikely that active immunization has occurred. Passively acquired anti-D rarely has an antiglobulin titer above 4 and should be entirely IgG. When in doubt about whether anti-D is passive or represents active immunization, it is always better to administer RhIG at the appropriate time. The crossmatches are compatible because D-negative RBCs would have been chosen for transfusion.

198.

D. The Kleihauer-Betke acid elution stain is used to quantify the amount of fetal cells present in the maternal circulation postpartum to calculate the correct dose of RhIG to administer. Adult hemoglobin is soluble in acid buffer, whereas fetal hemoglobin is resistant to acid elution. A thin blood smear is subjected to acid elution, pH 3.2, and then is stained with erythrosin B and Harris hematoxylin. Normal adult cells appear as pale ghosts microscopically; fetal cells are bright pink. The number of fetal cells in 2000 maternal cells is calculated. The volume of fetal hemorrhage is calculated as follows:

$$\frac{\text{Number of fetal cells}}{\text{Number of maternal cells}} \times \text{material blood volume (estimate 5000 mL)} = \text{fetal bleed}$$

This is equivalent to: Fetal cells expressed as a percentage of maternal cells × 50 = mL of fetal whole blood. One vial of a standard 300-μg dose protects the D-negative mother against sensitization to the D antigen for a 30-mL bleed. Therefore, the fetal hemorrhage volume is divided by 30 to determine the number of vials.

199.

B. Neither anti-P_1 nor anti-Le^a is likely to cause HDN. They are almost exclusively IgM antibodies (cannot cross the placenta), and the corresponding antigens are not well developed on neonatal red blood cells. Both anti-K and anti-c are almost exclusively IgG antibodies and are capable of causing serious HDN. However, the K antigen has a much lower frequency (<10%) in the population than the c antigen (>80%), so the infant is much more likely to be c+.

200.

D. The Kleihauer-Betke acid elution stain is used to estimate the amount of fetal red blood cells present in the circulation of a D-negative mother postpartum. Failure to quantify the FMH may result in the administration of insufficient Rh immune globulin. Sensitization to the Rh antigen may occur, leading to HDN in subsequent pregnancies. The fetal bleed is calculated using the formula: KB% × 50 = milliliters fetal blood present or 1.3 × 50 = 65 mL.

201.

C. One standard dose of Rh immune globulin (300 μg) protects the mother from a 30-mL bleed. Because the precision of a Kleihauer-Betke stain is poor, a margin of safety is employed to prevent RhIG prophylaxis failure. The total bleed in milliliters is divided by the level of protection in one dose (30 mL). For decimals less than five, round down and add one dose (e.g., 2.3 rounds down to 2 + 1 dose = 3 vials total dose); for decimals five or greater, round up and add one dose (e.g., 2.6 rounds up to 3 + 1 dose = 4 vials total dose).

Example:

$$\frac{65 \text{ mL bleed}}{30 \text{ mL}} = 2.2; \text{ give 3 vials RhIG}$$

202.

D. A positive autocontrol reacting at the antiglobulin phase of testing indicates that the patient has a positive DAT. All screening cells react at the same strength at the same phase of testing as the autocontrol. This indicates the presence of a warm (IgG) autoantibody, both on the patient's cells and in her serum. This patient's serum would be expected to react 4+ at AHG with all cells tested. Polyagglutinable cells will not react in the autocontrol, because the patient will lack the antibody corresponding to the antigen causing the polyagglutination. Rouleaux will not be present in the antiglobulin phase because all of the serum proteins are washed away before the AHG is added. A transfusion reaction may demonstrate a positive autocontrol and/or antibody screen; however, the reaction will be mixed field, because the patient's cells will not react with her alloantibody but the donor cells will react with the patient's antibody.

203.

A. The direct antiglobulin test (DAT) is the easiest and the quickest way to detect *in vivo* sensitization of red blood cells. The indirect antiglobulin test is used to detect *in vitro* sensitization of red blood cells and is most commonly used in antibody detection tests and crossmatching. Uses of the direct antiglobulin test include investigation of hemolytic disease of the newborn, autoimmune hemolytic anemia, transfusion reactions, and drug-induced sensitization of red blood cells.

204.

B. The only method that can be used to identify an antibody coating red blood cells is to perform an elution on those cells and then test the eluate against a panel. Autoabsorption of the patient's serum and then a panel on the autoabsorbed serum would be the method of choice for the identification of alloantibodies in her serum because she has not been recently transfused. Any panel on her serum would simply react with all panel cells tested because of the presence of the autoantibody.

205.

C. Most patients having warm autoimmune hemolytic anemia (WAIHA) respond well to steroids, which decrease the autoantibody production. Transfusing these individuals usually will cause the autoantibody to be produced in greater amounts. Transfusion in these cases is often counterproductive because the donor cells are destroyed as quickly as the patient's own cells and the expected increment in hemoglobin from the transfusion does not occur. Because this woman needs more oxygen-carrying capacity, transfusion with FFP will not be of help to her. Plasma exchange may remove antibody from her circulation but will not provide any oxygen-carrying capacity.

206.

D. Most antibodies in WAIHA have specificity that appears to be directed toward the Rh blood group system antigens. Sometimes the antibody has a simple specificity; anti-e is the most common warm autoantibody of simple specificity. Usually the antibody has a broad specificity, reacting with blood cells of most Rh phenotypes except for rare phenotypes such as Rh_{null}. In addition, there are mimicking or relative specificities—for example, an apparent auto anti-e that can be adsorbed onto e-negative red cells.

207.

C. Although it is best to avoid transfusing patients with warm autoantibodies, life-threatening anemia may develop, which necessitates them to receive blood. The primary concern in patients demonstrating serum warm autoantibody is to detect and identify the presence of underlying alloantibodies that may be masked by the reactions of the autoantibody. One must be certain to differentiate between auto- and alloantibodies. If the autoantibody appears to have a simple specificity such as anti-e, alloantibody identification can be accomplished by testing reagent red cells that are e-negative and antigen positive for the alloantibodies to be excluded. Alternatively, when sufficient e-negative panel cells are not available to do a thorough rule out, warm autoadsorption of the serum with the untransfused patient's red cells will remove autoantibody but not alloantibody. Alloantibodies will cause the transfused RBCs carrying the corresponding antigen to be removed from the recipient's circulation even more rapidly than the patient's own RBCs and will not provide the oxygen-carrying capacity the patient requires.

208.

A. Autoantibodies are directed against antigens present on the patient's own red blood cells. The easiest way to determine whether the anti-e is auto or allo when the patient has not been recently transfused is to type the patient's red cells with a low-protein anti-e reagent. A high-protein reagent should not be used, since a false positive reaction is likely to occur. The preparation of an eluate when patients have no history of recent transfusion is not necessary; it is presumed that the eluate contains only autoantibody.

209.

C. When a patient has been recently transfused, a mixed-field positive DAT may indicate the presence of clinically significant alloantibody attached to circulating donor's cells. A positive DAT may also reflect autoantibody attached to patient's or donor's cells, either because of drug sensitivity or secondary to clinical disease, which is less likely in this case. It is important to obtain information, including diagnosis, clinical condition, medication history, and laboratory data such as hematocrit, bilirubin, haptoglobin, and reticulocyte count, both pre- and posttransfusion to determine whether a delayed hemolytic transfusion reaction (DHTR) has occurred. A DHTR commonly demonstrates a mixed-field DAT result.

210.

C. The reaction in screening cell I is 2+. This reaction is characterized by the presence of some agglutinates in the bottom of the gel microtube along with antigen-antibody complexes that are distributed throughout the gel. The 2+ reaction represents agglutinates of many different sizes. This is different than a 1+ reaction in which all of the agglutinates are confined to the lower half of the gel microtube. Larger agglutinates are trapped at the top of the gel, and the smaller the agglutinates, the further they fall through the matrix.

211.

A. When centrifuged, unagglutinated cells, like those in SC II, fall completely through the gel matrix and form a pellet at the bottom of the microtube. The agglutinated cells in SC I have been trapped in the gel according to the size of the agglutinates. The gel matrix is porous and will suspend antigen-antibody agglutinates in the gel when they are too large to fall further through the matrix.

212.

C. Paroxysmal cold hemoglobinuria (PCH) is the rarest form of cold autoimmune hemolytic anemia. It is characterized by an IgG antibody that usually exhibits anti-P specificity. The anti-P attaches to the RBC during exposure to cold and then activates complement when the patient rewarms, causing hemolysis. A diagnostic test used to aid in the identification of PCH is the Donath-Landsteiner test. CHD is usually caused by anti-I, which is IgM.

213.

C. The Donath-Landsteiner (D-L) test is a diagnostic test for PCH. A characteristic of the antibody involved is that it will bind to red blood cells at cold temperatures; at 37°C it will cause red blood cell lysis. In the D-L test, a tube with the patient's serum and test cells is incubated at 4°C whereas a control tube is incubated at 37°C. After the 4°C incubation, the tube is placed in the 37°C incubator. After incubation, the tubes are centrifuged and examined for hemolysis. If there is hemolysis noted in the test and none in the control, the test is positive. If no hemolysis is noted in either tube, the test is negative, and if hemolysis is present in both tubes, the test is invalid.

214.

C. Cephalosporin antibodies will generally *not* react with red blood cells unless the RBCs have been treated with the drug. The negative antibody screening test indicates that crossmatches will be similarly compatible. An eluate from the patient's red cells would be expected to be nonreactive with untreated RBCs as well. An eluate would not be prepared unless the patient had been recently transfused and a DHTR was suspected.

215.

A. The drug α-methyldopa (Aldomet®), a blood pressure medication that is no longer frequently used, induces autoantibody formation that is indistinguishable from antibody produced in WAIHA. The antibody produced usually reacts with all panel cells tested. Some of the other drugs that induce autoantibody formation are L-dopa, procainamide, and some nonsteroidal anti-inflammatory drugs.

216.

C. Of the drugs listed, only penicillin has the observed DAT profile. IgG alone is also generally seen when α-methyldopa and cephalosporins are the implicated drugs. When phenacetin, quinidine, and tolmetin are the drugs in question, only C3 (complement) is usually detected on the patient's red cells. Antibody in the patient's serum and/or eluate will not react with reagent red cells unless the RBCs are first treated with penicillin. This occurs because the antibody produced by the patient reacts with epitopes formed by the penicillin coating the RBCs.

217.

D. Although the cold autoagglutinin is generally IgM, complement is usually the only protein detected on the red blood cells. The IgM antibody binds complement at lower temperatures in the extremities (such as fingers exposed to cold temperatures) and then dissociates when the red cells circulate. The polyspecific AHG is not required to have anti-IgM but must have anti-IgG and anti-C3d.

218.

C. Antibody directed against drugs may be suspected if patients present a positive DAT with a nonproductive eluate. The eluate and serum antibody may demonstrate reactivity only with reagent red cells coated with the appropriate drug and not with uncoated red cells. A current medication history and demonstration of eluate reactivity against the appropriate drug-coated red cells confirm the cause of the positive DAT. Drug-related antibodies are seldom clinically significant, and elaborate testing usually is not performed unless the patient shows symptoms of active hemolysis.

219.

D. Autoantibody(ies) directed against self-antigens may attach to a patient's own red blood cells *in vivo,* resulting in a positive DAT. A variety of elution techniques can remove this antibody from the red cells for testing. When a patient has not been transfused recently (within 3 to 4 months), it may be presumed that only autoantibody is present in the eluate. If recently transfused, donor's cells may be present in the patient's circulation. Alloantibody may be attached to donor cells, and autoantibody may be attached to patient's or donor's cells. When this is the case, alloantibody and autoantibody both could be recovered in the eluate.

220.

B. A patient with a history of having had anti-Jk^a in her serum must always be given Jk(a–) blood. Anti-Jk^a in the plasma often drops to below detectable levels of antibody in the absence of stimulus. However, if Jk(a+) cells were to be given to the patient, the antibody would quickly rebound to high levels and cause a delayed hemolytic transfusion reaction. It is difficult to procure e negative blood because ~80% of the population is e+. Add that to the need for Jk(a–) blood and the likelihood of finding suitable units drops. It is much more important to avoid transfusing against the alloantibody, anti-Jk^a, than the autoantibody. The autoantibody will destroy transfused cells at the same rate it is destroying the patient's RBCs; however, the alloantibody will destroy the transfused cells more rapidly.

221–228.

(221:B, 222:D, 223:B, 224:B, 225:B, 226:B, 227:A, 228:C)

Mothers who are not candidates to receive Rh immune globulin (RhIG) are (1) D-positive, (2) D-negative giving birth to a D-negative infant, or (3) D-negative who have produced alloanti-D. For purposes of determining RhIG candidacy, the weak D phenotype is considered to be D-positive. If the mother is D-negative and has not made anti-D, she must receive RhIG when giving birth to a D-positive or weak D phenotype infant. When only one of the infants in a multiple birth is D-positive and the other(s) is (are) D-negative, the mother is a candidate for RhIG. The presence of antibodies other than anti-D in the pre- or postpartum serum does not preclude a D-negative woman from receiving RhIG. When the infant's red cells are DAT+, it may be difficult to determine the correct Rh status. Because RhIG is a low-risk product, experts recommend a fail-safe approach and give RhIG when in doubt. Most obstetricians routinely inject RhIG at 28 weeks antenatally to prevent sensitization during a third-trimester fetal bleed. This injected anti-D is sometimes detectable postpartum and does not preclude additional administration of RhIG at childbirth. Whenever doubt exists as to the weak D status of the infant or the origin of the anti-D, postpartum RhIG should be administered. The preferred method for determining fetomaternal hemorrhage (FMH) is the rosette test, which is more sensitive than a microscopic weak D test. The rosette test will detect FMH of >10 mL. A positive test would show one or more rosettes per field when an enhancing medium is used. Additional testing must be performed to quantify the amount of FMH to prevent sensitization to the D antigen. The Kleihauer-Betke acid elution stain is the standard technique available to determine whether a bleed of more than 15 mL red cells (30 mL whole blood) occurred, necessitating additional 300-μg doses beyond the standard one-vial dose. Other methods include the enzyme-linked antiglobulin test (ELAT) and flow cytometry. The 50-μg dose of RhIG is used only when a female of 12-weeks gestation or less suffers a miscarriage. It is not intended for administration postpartum or for injection antenatally, either at 28 weeks or after amniocentesis.

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